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SULFUR ON ALKALI SOILS

European and American investigators have shown convincingly that elementary sulfur may be readily oxidized in the soil by microörganisms. Pure cultures of these microörganisms have not yet been isolated. Preliminary work done in the laboratories of the New Jersey Agricultural Experiment Station shows that the oxidation of elementary sulfur may be accomplished by certain types of bacteria, and possibly also by molds. There is reason to believe that inoculation with pure cultures of sulfofying bacteria may find a place in progressive methods of soil treatment.

Meanwhile, it is quite certain that elementary sulfur, used in small or large amounts may be transformed by microörganisms into sulfuric acid. It is not proposed to discuss at this time the value of sulfuric acid thus formed for modifying the soil reaction. It need only be said in passing that the application of elementary sulfur has been found to be of great service in checking the development of scab on potato tubers. In a recent contribution to SOIL SCIENCE from the New Jersey Station, it was also shown that sulfur oxidation in soil may be made to play an important part in the utilization of insoluble phosphates by crops. Work now in progress has already disclosed a number of problems of great scientific and practical significance.

The investigations of C. B. Lipman, of the University of California, on the value of sulfuric acid in the reclamation of certain alkali soils would justify the suggestion that elementary sulfur could be used to great advantage instead of sulfuric acid as proposed. As is well known, there are vast areas of land in which carbonates and bicarbonates of sodium are present in sufficient amount to interfere partially or completely with profitable crop production. It is hoped, therefore, that experiments will be undertaken with sulfur as a means of reclaiming land which now contains an excess of these carbonates and bicarbonates.

JACOB G. LIPMAN.

August 24, 1916.

PHYSIOLOGICAL BALANCE OF NUTRIENT SOLUTIONS FOR PLANTS IN SAND CULTURES[1]

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ABSTRACT

The experiments described in this paper were conducted for the purpose of studying the relative growth rates of young winter wheat seedlings, when grown in a substratum of sand and supplied with a nutrient solution of the same initial total concentration, but varying in the proportions of the component salts. An initial total concentration of 1.75 atmospheres maximum osmotic pressure was employed for 36 different proportions of the three component salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$.

The series included all of the possible proportions of the three salts, when the components are made to vary by increments of one-tenth of the total possible osmotic pressure. Each culture consisted of 6 plants growing in washed quartz sand. The enameled steel pots employed were approximately 12 x 12 cm., inside diameter, and held 1500 gm. of dry sand. After the seedlings were planted the surface of the sand was covered with a thin layer of wax to prevent loss of moisture by evaporation. The solutions were renewed every three days by the addition of 250 c.c. of fresh solution through a funnel which occupied a position at the center of the pot. At the same time that the fresh solution was being added at the top, the old solution was removed from the bottom of the pot by means of suction applied to a small tube connecting with the interior. The total growth period was 24 days, during which time the total water loss from each culture was determined at the end of each 3-day interval. At the end of the growth period the cultures were compared with respect to: (1) dry weight of tops, (2) dry weight of roots, (3) total water loss, (4) water requirement per gram of dry tops, (5) water requirement per gram of dry roots, and (6) the ratio of the weight of tops to dry weight of roots.

Three preliminary series of wheat cultures were grown in sand and supplied with nutrient solutions having a range in initial total concentration from 0.2 to 5.0 atmospheres. One of these series was characterized by having 5 tenths of the total osmotic concentration derived from monopotassium phosphate, 2 tenths from calcium nitrate, and 3 tenths from

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magnesium sulphate, while the other two had 4 tenths of the total osmotic concentration derived from KH_2PO_4, 3 tenths from $Ca(NO_3)_2$, and 3 tenths from $MgSO_4$. These three series were in accord in showing that a total concentration of 1.75 atmospheres was well within the range required for optimal growth of wheat tops.

The main results may be summarized as follows:

1. The graphs representing the growth rate of young wheat plants, for three preliminary series, show a region of optimal growth rate lying between the concentrations 1.0 and 2.0 atmospheres.

2. With the initial total concentration about 1.75 atmospheres, the nutrient solution that produced the greatest dry weight of tops also produced the greatest dry weight of roots. This solution is characterized by having 2 tenths of the total osmotic concentration derived from KH_2PO_4, 7 tenths from $Ca(NO_3)_2$, and 1 tenth from $MgSO_4$.

3. A general comparison of the results from this sand culture series with solution cultures (Shive's) grown from the same lot of seed but at a different time period brings out some interesting comparisons, which may be summarized as follows: (1) the average dry weights of both tops and roots were decidedly greater for the plants grown in the sand than for those grown in the solutions, (2) the results obtained in the solution culture series having a total osmotic concentration of 0.1 atmosphere, are more nearly like those from the sand series than are the results secured from the more concentrated solution series (1.75 atm.) in which the solutions were of the same total osmotic concentration as that employed for the sand cultures, and (3) there is a marked difference between the solutions producing the best development of plants in sand and those giving the best growth in the solution cultures, with respect to the osmotic proportions of the three salts employed.

4. A comparison of the results from these two series, the one grown in solution and the other in sand cultures, furnishes evidence for the conclusion that selective adsorption plays an important rôle in bringing about the observed physiological differences.

5. The sand culture solutions giving *low* yields of tops are characterized by a *wide* range in the Mg/Ca ratio; a very wide range in the Mg/K ratio, and a narrow range in the Ca/K ratio value. The solutions giving *high* yields of tops show a *narrow* range in the Mg/Ca ratio; and a comparatively wide range in both the Mg/K and Ca/K ratio values.

6. The data presented support the conclusion of earlier workers to the effect that the total transpirational loss from a plant culture is approximately proportional to the growth made by the plants during the period of time considered.

7. The water requirement per gram of dry tops varies considerably with the different proportions of the component salts. From these data it appears that *low* water requirement for tops is associated with a *low*

partial osmotic concentration of mono-potassium phosphate, and that *high* water requirement is associated with *high* partial concentrations of both magnesium sulphate and mono-potassium phosphate.

8. The water requirement per gram of dry roots is much higher than the same value for tops.

9. A consideration of the ratio of tops to roots brings out the fact that in every instance a high water requirement corresponds to a high ratio of tops to roots.

10. Good growth of tops was found to be associated with a high osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ and poor growth of tops with a low value of this ratio. We are not justified, however, in drawing, from these results, any definite conclusions with respect to the calcium-magnesium ratio as such, since much of the superior growth, in the cultures where $Ca(NO_3)_2$ was in excess, may be ascribed to the presence of a large amount of the NO_3 radical, which is known to be favorable to very vigorous vegetative growth.

Introduction

During the closing years of the seventeenth century Woodward (46) grew spearmint, potatoes and vetch in rain, spring, river, conduit and distilled water, for the purpose of determining whether it was the water or the solid soil particles which nourished plants. It appears, however, that water cultures were not employed for the purpose of studying plant nutrition to any great extent until about 1859, at which time Knop (16) and Sachs (32) began their investigations of Liebig's theory that the materials dissolved in the soil water are not generally sufficient for plant growth and that plants derive food directly from the soil particles.

This early work of Knop and of Sachs, together with their subsequent experiments along the same lines, gave such an impetus to water-culture work that there has grown up, during the past half century, a very extensive literature upon the subject of water or solution cultures. Most of the earlier publications in this field are to be found in *Die Landwirtschaftliche Versuchs-Stationen*, while a general review of the literature may be found in such works as those of Pfeffer (30), Duggar (9), and Czapek (7). The very recent work of Tottingham (40) and the publications of Shive (37, 38) have added a very important and interesting chapter to the already voluminous literature upon the subject of plant nutrition with special reference to the physiological requirements of the plant.

After an extensive chemical study of the components of a standard formula, Tottingham grew preliminary cultures of wheat in two forms of Knop's solution, one including mono-potassium phosphate and the other having the phosphate in the di-potassium form. In these cultures, the solution containing the mono-potassium phosphate produced 17.8

per cent better growth of tops and 17.5 per cent better growth of roots than the solution containing the di-potassium salt. Following this preliminary work, Tottingham employed 84 different solutions, all of approximately the same total osmotic concentration, but each culture differing from all of the others in the proportions of the four salts, mono-potassium phosphate, potassium nitrate, calcium nitrate, and magnesium sulphate. To each of the cultures was added the usual trace of iron, in the form of ferric phosphate. With a total concentration at about the optimum for young wheat seedlings, the solution having the proper salt proportions to give the best growth of tops was found to produce an improvement of 11 per cent, based on the dry weight of tops grown in Knop's solution of the same total osmotic concentration.

Repeating some of these tests, Shive obtained results that show a very close agreement with those previously reported. His best growth was secured with the same proportions of salts as those found best by Tottingham, and an improvement of 12 per cent over Knop's solutions was obtained.

As a result of the further study of these four-salt solutions, Shive has been able to make a combination of three nutrient salts which contain all of the essential elements of plant growth (with the exception of iron) and which does not form a precipitate in solutions of the required concentration. The solutions employed by this writer contain mono-potassium phosphate, calcium nitrate and magnesium sulphate, and differ from the four-salt solutions just mentioned by omission of potassium nitrate. The three salts dissociate in dilute solutions, to form all of the ions that are found in the four-salt mixture of Knop or Tottingham. Testing this three-salt solution by the same general method as was employed by Tottingham, Shive secured cultures showing an improvement of 27 per cent over Knop's solution of the same total concentration. With this series of wheat cultures was included Tottingham's best solution, which showed a corresponding increase over Knop's solution of but 16 per cent.

Upon learning of these recent results, the writer became greatly impressed with the desirability of studying the effect of these solutions upon plants grown in sand where some of the physical environmental conditions of the soil are present, but where the cultures are relatively unaffected by the biological complications introduced when ordinary soils are used. The work of Tottingham and that of Shive were confined exclusively to water cultures, in which the solution was renewed at frequent intervals. Accordingly, it was planned to repeat a part of the work of Shive, using the same three-salt solution as was employed by him and the same kind of plant (wheat), but employing pure sand as the substratum, instead of having the roots of the plants completely immersed in the free solution. In order to secure a renewal of the nutrient solu-

tions at intervals during the growth of the plant, a special method (25) was devised, whereby the old nutrient solution could be removed and fresh solution added to the pots, without seriously disturbing the relation between the roots and the sand.

This investigation was conducted in the Laboratory of Plant Physiology of the Johns Hopkins University, under the direction of Dr. B. E. Livingston, to whom the author is deeply indebted for many criticisms and helpful suggestions.

METHODS

I. Sand Cultures with Renewed Solutions

After a great deal of preliminary work, the following method was adopted as best suited to the needs of the experiment. The pots used were of enameled steel ("graniteware"), approximately 12 x 12 cm., inside diameter, narrowing slightly toward the base and having a wide projecting rim at the top. When filled to within about 3 cm. of the top, these pots hold 1500 gm. of dry quartz sand. To provide for the removal of the solution a small lead tube is soldered into the side as near the bottom as possible.[1] The soldered joint and the lead tube are covered with paraffin, to guard against possible lead-poisoning, and the outlet of the tube closed by means of a short length of rubber tubing provided with a pinch-cock. The accompanying photograph, Plate I, shows the form of the pot and gives a good general idea of the appearance of the cultures at a period of about 20 days after planting. The description of the method given in the following paragraphs includes the details of manipulation from the starting of the seedlings to the harvesting of the plants.

The seed is soaked in water and the seedlings grown, in the manner described by Tottingham, to a height of about 3 or 4 cm., when they are ready to be transferred to the sand cultures. While the seed is being germinated, 1500 gm. of dry quartz sand (previously washed several times with distilled water) are weighed into the pot, the outlet at the bottom of the pot being screened on the inside by means of a plug of glass wool inserted before the pot is filled. With the pinch-cock closed, distilled water is now added to the pot until the sand is completely saturated, after which the pinch-cock is opened and the surplus water allowed to drain out through the tube at the bottom of the pot, until the last free water has disappeared from the surface of the sand. An inverted hemispherical porcelain funnel is placed in position at the center of the soil surface, as shown in Plate I, and the pot is then ready to receive the seedlings.

After careful selection for uniformity, the seedlings (six in number)

[1] In order to make the hole in the side of the pot it is necessary first to chip off a small piece of enamel with the sharp point of a file. This serves to give entrance to the point of a small twist drill which then passes through the iron and chips off the enamel on the inside, thus exposing sufficient iron to give adherence to the solder.

are planted, being equally spaced on a circle lying midway between the edge of the funnel and the wall of the pot. Care is taken to place the seedlings at such depth that the top of the grain is just level with the surface of the sand. After all of the seedlings are in place, the pinch-cock is closed, and the pot is tapped gently on the table until free water appears on the surface of the sand. This manipulation serves to pack the sand around the roots of the seedlings and at the same time to level the surface of the sand preparatory to putting on the seal of Briggs and Shantz (4) wax. This wax is composed of 80 per cent paraffin and 20 per cent petrolatum, the exact proportions being unimportant. The mixture has such a low melting point and is such a poor heat conductor that it can be poured around the most delicate seedlings without injury.[1] The surplus water is then drawn out of the pot by application of suction (by means of a water-aspirator) to the tube at the bottom, and a thin layer of the melted wax is flowed over the surface, completely covering the sand between the funnel and the wall of the pot. Care should be taken to have the wax only a few degrees above its melting point or the seedlings may be injured at the point of contact with the wax. The surface must be sealed to prevent the loss of water by evaporation from the surface of the sand, and, of course, the walls of the pot must be impervious to moisture in order that transpiration can be measured and the concentration of the nutrient solution controlled.

The pot is now ready to receive the nutrient solution, which is added through the funnel at the top while the water is being removed at the bottom by the application of suction to the outlet tube. A double or triple portion of the nutrient solution is passed through the sand at this first application, in order to flush out the distilled water. The pot is now placed on the balance and the removal of solution is continued until the sand has been reduced to the desired moisture content, which should be, as nearly as possible, the optimum for plant growth. At the end of each 3-day period the pot is again weighed, and sufficient water is added through the funnel to bring the entire system back to its original weight. A fresh nutrient solution is then added (250 c.c. for pots of this size), while an equivalent quantity of solution is removed at the bottom. A nutrient solution of the same concentration may be used throughout the entire period of growth, or it may be varied from time to time as the plants continue to develop. The plants may be harvested at any time by removing the wax seal and cutting them level with the surface of the sand. If desired, the roots may be recovered from the sand by washing them out with a jet of water. The records of pot weights give the amount

[1] The writer has found the paraffin sold under the trade name "Parawax" to be cheap and very satisfactory. Care must be taken to secure a good grade of petrolatum or vaseline. Some brands seem to contain volatile substances which cause injury to the plants at the point of contact with the seal. The Chesebrough brand of white vaseline has been found to be safe to use for this purpose.

of water lost by each culture (transpiration) and the harvest records may be made to include the dry weights of both tops and roots.

II. Materials Used

The substratum used in these cultures consisted of medium-fine white sand,[1] which had been previously washed four times with distilled water from a Barnstead still. For the first washing a 2-gallon glazed stoneware jar was filled about two-thirds full of distilled water and the dry sand slowly poured in while the contents of the jar were kept agitated by means of a large glass stirrer. The surplus water was then decanted, after which the sand was spread out on large sheets of paper until air-dry. The dry washed sand was then weighed into the granite-ware pots and was afterwards washed three times by covering with distilled water and drawing the water through the material by means of suction applied to the tube at the bottom of the pot. Failure of control cultures to develop in the sand supplied only with distilled water, instead of with the nutritive solution, gave conclusive proof that this washing treatment was sufficient to remove any nutrient salts that might have been in the unwashed sand.

The salts used in making up the culture solutions were Baker's "analyzed" mono-potassium phosphate and calcium nitrate and Merck's "blue label" magnesium sulphate. Stock solutions were prepared by dissolving gram-molecular weights of the salts separately in Jena flasks, each solution being made up to a volume of one liter. Before making up the final nutrient solutions the stock solutions were diluted to one-fourth molecular and stored in flasks, each of the latter being connected to a burette with automatic filling arrangement. By means of these burettes the required amounts of solution were drawn at each time when a new set of nutrient solutions were to be prepared. The drying of the salts, the making up of the stock solutions, and all of the other manipulations with respect to the making up of the nutritive solution were substantially the same as those described by Shive (38).

III. Culture Solutions

The growth-rate of a plant is determined by two sets of conditions, one of which is internal to the plant and hence thus far very largely beyond our control, while the other is external, or environmental, and hence subject more or less to artificial control. In the present work an attempt

[1] A mechanical separation of this sand gave the following percentages of different sized particles:

Fine Gravel (2.0 to 1.0 mm.)	Coarse Sand (1.0 to 0.5 mm.)	Medium Sand (0.5 to 0.25 mm.)	Fine Sand (0.25 to 0.1 mm.)	Very Fine Sand (0.1 to 0.05 mm.)
0.14	48.62	26.40	22.88	1.46

was made to have the internal conditions of the various plants as nearly uniform as it was possible to make them, by starting a large number of seedlings and selecting plants of uniform size and appearance. The environmental conditions may be further divided into two groups, one of which may be defined as aerial and the other as subterranean. Since the present study concerns the subterranean environment it was essential that the aerial environment should be made as uniform as possible for all of the cultures. This uniformity in aerial conditions is best secured by placing the cultures on rotating tables, as described by Shive, thus exposing all of the plants to approximately similar changes of heat, light, and moisture conditions. However, since rotating tables were not available for this work, a less convenient method was employed: the cultures were shifted in position each day, in regular order, on a stationary table. In order to avoid unequal shading, as far as possible, the cultures were placed in two single rows extending east and west in the greenhouse, the rows being of sufficient distance apart so that at no time during the day was there any shading of one row by the plants in the other. As a further precaution, each row of pots stood on narrow slabs of slate which were elevated about 15 cm. above the general level of the table.

In studying the comparative physiological effects of different nutrient solutions in such cultures as these, it is of course desirable to have uniformity in all of the subterranean conditions affecting the plant, excepting those conditions that are dependent upon the properties of the nutrient solutions, but this is difficult of accomplishment. A fairly satisfactory degree of uniformity in the subterranean physical conditions was secured by filling all of the pots from the same bulk sample of sifted sand and by taking care to maintain the same amount of moisture in all of the pots throughout the duration of the experiment. As has been pointed out by Livingston (18) and others, the cultural solution may influence the plant in two different ways. The chemical effect of the solution is dependent upon the chemical nature of the salts present and also upon the relative amounts of the different salts contained in the nutrient solution. On the other hand, the solution may exercise a marked influence upon plant growth in a purely physical way, by virtue of its total concentration to which is related the osmotic equilibrium between the nutrient solution outside the roots and the cell sap within. When water cultures are employed, the total concentration of the solution with which the roots are in contact is known with a fair degree of accuracy, but in sand cultures the solution may undergo a change not only in its total concentration but also in the relative proportions of the different salts, as the result of its contact with the solid particles of the substratum.

Elaborate investigations concerning the relation between the concentration of the solution in the soil and the growth of plants have resulted in disappointment largely because of the fact that, while it has been admitted that the adsorbed layer at the immediate surface of the soil grain

is of different concentration from that of the mass of free solution, it appears that no method has yet been devised for determining this difference in concentration. Furthermore, no direct experimental evidence has been reported that would throw light upon the question of the availability and the non-availability of salts in the adsorbed layer. It has been suggested that the thickness of the adsorbed layer is frequently less than that of the outer cell walls that cover the absorbing protoplasm in epidermal cells of roots. From this, it is argued that, since the protoplasm does not come into direct contact with the adsorbed layers about the soil grains, this layer, therefore, must be unavailable to the plant except for the possible slow diffusion of the salts from the adsorbed layer out into the adjacent free-water film. As a further support for the theory that the adsorbed layer is not available to plants, it is pointed out that the addition of a few pounds of a soluble potash salt to an acre foot of soil, for instance, often produces a very decided increase in the growth of the crop, although there may be present in the upper foot of soil as much as 10,000 pounds of potassium. This is interpreted to mean that the salt originally present in the soil solution was practically all in the adsorbed layers and that the relatively slight addition resulted in increasing the concentration of the free solution.

The effect of adsorption in reducing the toxicity of certain dissolved substances has been observed by several investigators.

True and Oglevee (41) studied the effect of the addition of difficultly soluble substances to toxic electrolytes and non-electrolytes. Sand, filter paper and paraffin were added to dilute solutions of the toxic substances and the changes produced by the addition of these materials were detected by observing the growth rate of the primary root of *Lupinus albus* when emersed in the different solutions for a period of 24 hours. The presence of these insoluble substances in the toxic solutions always gave an accelerated growth rate, the effect being quite similar to that produced by simple dilution. These investigators regard it as probable that the substances added to the toxic solutions acted as adsorbing surfaces for the molecules or the ions of the toxic substances dissolved in the liquid. This would affect the solution much like the addition of water, to bring about a decreased number of molecules or ions in a given volume of free solution. Similar results were obtained by Breazeale (2).

In a paper under the same title published the following year, these last mentioned authors (42) cite the work of Nägeli, with *Spirogyra* growing in distilled water obtained from copper containers. Nägeli (27) had found that his solutions, containing minute traces of a toxic metal, could be made harmless by the addition of paraffin, graphite, filter paper, or glass.

Dandeno (8) has also studied the effect of the addition of finely divided soils to toxic solutions. He found that the effect of the addition of non-chemical bodies to toxic solutions very much retarded the action

of the toxic substances in bringing death to the radicles of plants growing in such solutions.

Jensen (15) has shown that the introduction of pure quartz flour into a toxic solution reduces its toxicity to a marked degree. He states, however, that it is an open question whether the reduction in toxicity is due (1) to adsorption, (2) to reduced freedom of movement of the solute particles (that is, a reduction of the diffusion rate), or (3) to possible chemical changes induced by the presence of the finely divided quartz.

Breazeale (1) quotes some of Livingston's unpublished data, to show that in soil or in sand cultures the effect of concentration is quite different from that found in water cultures. These data indicate that the concentration best suited to the growth of wheat in water cultures is about 300 parts per million, while in sand cultures the solution giving the best growth rate has an initial concentration of approximately 2500 parts per million.

The present investigation furnishes some very important evidence concerning the availability of the adsorbed salts, which evidence will be discussed after the experimental results have been presented.

Following the nomenclature employed by Tottingham (40) and Shive (38), the concentrations used will be expressed in terms of osmotic concentration (maximum osmotic pressure in atmospheres[1]), or in terms of gram-molecules per liter of solution.

Experimentation

I. Determination of the Optimal Total Concentration

Working with water cultures, Tottingham (40) secured his best growth of wheat seedlings in a solution having a total osmotic concentration of 2.50 atmospheres, but he calls attention to the fact that this concentration may be somewhat above that required for optimal growth, and Shive (38) has shown that a total concentration of 1.75 atmospheres of maximum osmotic pressure lies within the range of concentration required for the best growth of wheat seedlings.

To determine if these approximately optimal total concentrations, found for water cultures, would hold good for sand cultures, three preliminary series of sand cultures were grown. The cultures of each series all received solutions having the same proportions of the three component salts, but the total osmotic concentration of the solution used was different for the various individual cultures in the same series. These preliminary cultures will be designated as Series A, Series B, and Series C. Series A and B were grown simultaneously from April 9 to April 29, while Series C was started April 28 and harvested May 18. Each series consisted of 6 cultures, differing from each other, as has been mentioned, in the total concentration of the nutrient solution employed, but all cultures

[1] For a discussion of the methods used for measuring the physical properties of solutions, and an explanation of the terms employed, see Findlay, Alexander (10), Washburn, E. W. (43).

in the same series having the same salt proportions. The solutions for Series A ranged in concentration from a minimum of 0.5 of an atmosphere to a maximum of 3.0 atmospheres, and were further characterized

TABLE I

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.5 TO 3.5 ATMOSPHERES

Series A, conducted from April 9 to April 29, 1915

Culture No.	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
1	0.5	5.1	1.5	4.3	0.8022	1.00
2	1.0	10.2	3.0	8.6	1.0498	1.31
3	1.5	15.3	4.5	12.9	1.0998	1.37
4	2.0	20.4	6.0	17.2	1.2454	1.55
5	2.5	25.5	7.5	21.5	1.0006	1.25
6	3.5	35.7	10.5	30.1	0.8678	1.08

by having 5 tenths of the total osmotic concentration derived from mono-potassium phosphate, 2 tenths from calcium nitrate, and the remaining 3 tenths from magnesium sulphate. The solutions employed in Series B

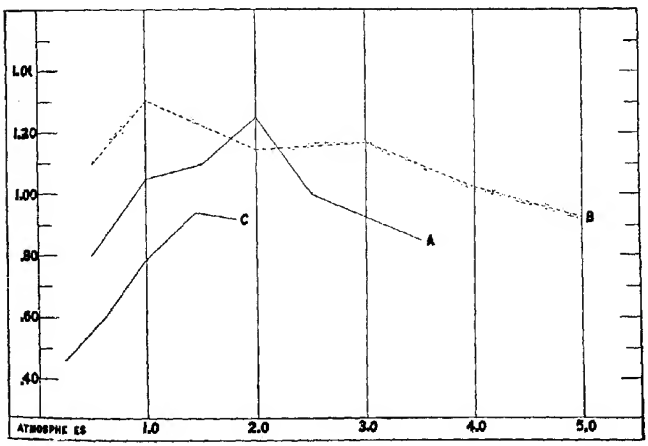

Fig. 1.—Dry weights of wheat grown for 20 days in sand cultures, with a three-salt solution varying from 0.1 atmosphere to 5.0 atmospheres total osmotic concentration.

had a range in total concentration from a minimum of 0.5 of an atmosphere to a maximum of 5.0 atmospheres, and derived 4 tenths of the total concentration from mono-potassium phosphate, 3 tenths from calcium

nitrate, and 3 tenths from magnesium sulphate. In Series C the solution ranged in total concentration from 0.2 of an atmosphere to 1.8 atmospheres, with the relative proportions of the three salts the same as in Series B. These particular sets of salt proportions were selected for the preliminary series because of the fact that Shive had already shown that these are associated with high yields of tops in solution cultures.

The data concerning the yield of tops in these preliminary series are given in Tables I, II and III. In these tables the first column gives the culture numbers; the second column shows the total concentration of the solutions employed, stated (in terms of maximum osmotic pressure) as atmospheres or fractions of an atmosphere. The three succeeding columns show, in each instance, the volume of stock molecular solution necessary for a liter of the required nutrient solution. Then follow two columns, one of which is devoted to the absolute, and the other to the relative dry weights of tops, the latter expressed in terms of Culture 1,

TABLE II

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.5 TO 5.0 ATMOSPHERES

Series B, conducted from April 9 to April 29, 1915

No. Culture	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
1	0.5	4.6	2.5	5.3	1.1074	1.00
2	1.0	9.2	5.0	10.6	1.3347	1.21
3	2.0	18.4	10.0	21.2	1.1430	1.03
4	3.0	27.6	15.0	31.8	1.1749	1.06
5	4.0	36.8	20.0	42.4	1.0210	.92
6	5.0	46.0	25.0	53.5	0.9256	.84

taken as unity. The relative dry weights obtained in these series are shown in the graphs of figure 1. From an inspection of the graphs it will be seen that (with the osmotic proportions of the three salts here employed) the best growth of tops in sand cultures was obtained by the use of nutrient solutions with total concentration between 1 and 2 atmospheres. Since the concentration of Shive's optimal water-culture solution (1.75 atm.) is within the range of optimal concentrations as shown by these preliminary sand cultures, that concentration was employed in the subsequent work here to be reported.

II. *Determination of the Effect of Thirty-six Different Salt-Proportions, with the Total Concentrations and Other Conditions Alike*

Following the preliminary work recorded in the previous section of this paper, wheat plants were grown in a complete series of sand cultures, to each pot of which was added, at 3-day intervals, a three-salt nutrient solution. The method by which the old solution was withdrawn and the fresh solution added to the pots has already been described. In this series, 36 cultures were employed, each of which received at the end of successive 3-day periods a culture solution having a total osmotic concentration of 1.75 atmospheres. The solution supplied to each particular culture differed, however, from that supplied to the other cultures in the series, with respect to the proportions of the three main component salts, monopotassium phosphate, calcium nitrate, and magnesium sulphate.

TABLE III

DRY WEIGHTS OF TOPS OF WHEAT GROWN FOR 20 DAYS IN SAND, WITH THREE-SALT SOLUTIONS VARYING IN TOTAL CONCENTRATION FROM 0.2 TO 1.8 ATMOSPHERES

Series C, conducted from April 28 to May 18, 1915

Culture No.	Total Concentration of Solution	Amount of Molecular Solution Required per Liter of Nutrient Solution			Dry Weights	
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Absolute	Relative to Culture 1
	atm.	c.c.	c.c.	c.c.	gm.	
[1]0A	H_2O	....	....	....	.1547	....
0B	H_2O	....	....	....	.2260	....
1A	0.2	1.8	1.0	2.1	.4580	[2]1.00
1B	0.2	1.8	1.0	2.1	.4526	
2A	0.6	5.4	3.0	6.3	.6234	[2]1.32
2B	0.6	5.4	3.0	6.3	.5804	
3	1.0	9.0	5.0	10.5	.7910	1.73
4	1.4	12.6	7.0	14.7	.9368	2.06
5	1.8	16.2	9.0	18.9	.9180	2.00

[1] Culture number 0 received only distilled water in this series. Cultures 0, 1 and 2 were in duplicate, each pair being indicated by A and B.

[2] Mean from two cultures.

The method of calculation by which the partial osmotic concentration and the volume-molecular concentration of each component salt in mixtures such as this, with a fixed total osmotic concentration, has been discussed by Tottingham (40, p. 177-182, 192), in connection with his four-salt solutions. In calculating the amount of each salt required to produce the total concentration required by the series (1.75 atm.) it was assumed that the degree of ionization of each salt is independent of the presence of the other two salts. In other words, the assumption was here made that each of the three salts would behave, in the presence of the other two, in the same manner as it would if dissolved in distilled water. The lowering of the freezing-point was determined by Shive (36) for each

one of the three-salt solutions employed by him, and it appeared from these determinations that the freezing-point lowerings were approximately the same for all of the solutions of his optimal series. Since Shive's series of optimal solutions are the ones here employed, it may safely be concluded that the *initial* total osmotic concentration of the nutrient solutions used in these sand cultures very closely approximated 1.75 atmospheres.

Table IV gives the volume-molecular concentrations of each salt required to produce from 1 tenth to 8 tenths of the total osmotic concentration for the various solutions in the series under consideration, these being taken from Shive's Table I (36, p. 339). To determine the volume molecular partial concentration of any given salt in any solution of this series it is only necessary to find, in the first column of this table, the number of tenths of the total concentration to be assigned to that particular salt, and then to take from the proper column the volume molecular concentration given opposite this number.

TABLE IV

VOLUME-MOLECULAR PARTIAL CONCENTRATIONS REQUIRED TO PRODUCE FROM FROM 1 to 8 TENTHS OF THE TOTAL OSMOTIC CONCENTRATION FOR A SERIES OF SOLUTIONS HAVING A TOTAL CONCENTRATION OF 1.75 ATMOSPHERES

Tenths of Total Concentration	Partial Concentrations in Gram Molecules per Liter		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1	0.0036	0.0026	0.0050
2	0.0072	0.0052	0.0100
3	0.0108	0.0078	0.0150
4	0.0144	0.0104	0.0200
5	0.0180	0.0130	0.0250
6	0.0216	0.0156	0.0300
7	0.0252	0.0182	0.0350
8	0.0288	0.0208	0.0400

For convenience in designating the individual cultures and to give clearness to the following discussions, the cultures may be arranged on an equilateral triangular diagram, as was done by Shive for his similar series. This diagram is shown in figure 2, and in another form in figures 3, 5 and 8. Similar graphic schemes have been used extensively in chemical interpretation, and have been employed by Schreiner and Skinner (33, 34) and by Tottingham (40), as well as by Shive (38) and by Harris (13). The individual cultures are represented by circles, and it will be observed that the diagram has eight rows, the lower one of which contains eight individual cultures. Proceeding upward each row has one culture less than the one below it, and the eighth row contains but a single culture. The employment of shaded segments to the various partial osmotic concentrations of the three salts, in each of the 36 cultures under consideration, is an adaptation of the scheme employed by Harris (13) in his study of the alkali salts in soils. The unshaded segment in each cir-

cle represents the number of tenths of the total osmotic concentration derived from calcium nitrate; the segment marked by small crosses represents the number of tenths derived from mono-potassium phosphate; and the stippled segment indicates the number of tenths due to magnesium sulphate. The system of numbering the individual cultures, and the corresponding nutrient solutions, is the same in figure 1 as that employed by Tottingham (40, p. 194) and by Shive (38, p. 341). Proceeding from the base to the apex of the triangle, the rows are numbered from R1 to R8, while the individual cultures in each row are numbered from left to right. For example, the fourth culture from the left in the second row from the base is designated R2 C4, and similarly the second culture in the seventh row is R7 C2.

The diagram of figure 2 shows that all of the solutions represented as in the first row have approximately 1 tenth of their total osmotic concentration from mono-potassium phosphate, those in the second row 2 tenths, this amount increasing by increments of 1 tenth from row to row, until the apex of the triangle is reached, at which point the single culture in row 8 has 8 tenths of its total concentration due to mono-potassium phosphate. As indicated by the shading, the first culture, at the *left* in each row, has 1 tenth of its total concentration due to calcium nitrate, and this partial concentration increases regularly by increments of 1 tenth until the opposite side of the diagram is reached. In a similar manner the osmotic partial concentrations of magnesium sulphate increase from *right* to *left* in each row. The circle occupying the position R1 C2 has 1 tenth of its total area unshaded, 2 tenths marked by crosses, and the remaining 7 tenths stippled, thus indicating that the solution used for this culture had the osmotic proportions of 1 tenth mono-potassium phosphate, 2 tenths calcium nitrate and 7 tenths magnesium sulphate. Throughout this paper the individual cultures will be designated by the row number and by the position occupied in the row, using the nomenclature employed by previous writers. The partial volume-molecular concentration of each salt in each of the 36 solutions is given in Table V, together with the corresponding values of the three cation ratios. Each solution has a total osmotic concentration of 1.75 atmospheres.

Discussion of Results

I Introductory

The series of cultures which are now to be considered were grown in sand for a period of 24 days extending from May 15 to June 8, 1915. The wheat used was of the Fulcaster variety, from the same lot as was used by Tottingham (40) and by Shive (38), in their water cultures. As has already been stated, the methods employed for the germination of the seed and for the manipulation of the solutions were the same as those described by Shive (38), with such modifications as were made necessary by the employment of sand instead of water cultures. A detailed ac-

count of the method employed in the preparation of the sand and the manipulation used to secure a renewal of the solution at regular intervals has already been given.

TABLE V

PARTIAL VOLUME-MOLECULAR CONCENTRATION OF EACH OF THE SALTS IN EACH OF THE 36 THREE-SALT SOLUTIONS EMPLOYED FOR WHEAT IN SAND CULTURES; ALSO, THE THREE VALUES OF THE CATION RATIOS FOR EACH SOLUTION

Total concentration of each solution 1.75 atmospheres

Solution Number	Partial Solutions in Gram-Molecules per Liter			Cation Ratio Values[1]		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Mg / Ca	Mg / K	Ca / K
R1 C1	.0036	.0026	.0400	15.40	11.10	0.72
C2	.0036	.0052	.0350	6.74	9.72	1.44
C3	.0036	.0078	.0300	3.85	8.34	2.16
C4	.0036	.0104	.0250	2.40	6.94	2.88
C5	.0036	.0130	.0200	1.54	5.55	3.60
C6	.0036	.0156	.0150	0.96	4.17	4.33
C7	.0036	.0182	.0100	0.55	2.78	5.04
C8	.0036	.0208	.0050	0.24	1.39	5.77
R2 C1	.0072	.0026	.0350	13.46	4.86	0.36
C2	.0072	.0052	.0300	5.77	4.17	0.72
C3	.0072	.0078	.0250	3.21	3.47	1.08
C4	.0072	.0104	.0200	1.92	2.77	1.44
C5	.0072	.0130	.0150	1.15	2.08	1.80
C6	.0072	.0156	.0100	0.64	1.38	2.16
C7	.0072	.0182	.0050	0.27	0.69	2.52
R3 C1	.0108	.0026	.0300	11.53	2.78	0.24
C2	.0108	.0052	.0250	4.81	2.32	0.48
C3	.0108	.0078	.0200	2.53	1.85	0.72
C4	.0108	.0104	.0150	1.44	1.39	0.96
C5	.0108	.0130	.0100	0.77	0.98	1.20
C6	.0108	.0156	.0050	0.32	0.46	1.44
R4 C1	.0144	.0026	.0250	9.61	1.74	0.18
C2	.0144	.0052	.0200	3.85	1.39	0.36
C3	.0144	.0078	.0150	1.92	1.04	0.50
C4	.0144	.0104	.0100	0.96	0.69	0.72
C5	.0144	.0130	.0050	0.38	0.35	0.90
R5 C1	.0180	.0026	.0200	7.69	1.10	0.14
C2	.0180	.0052	.0150	2.88	0.83	0.29
C3	.0180	.0078	.0100	1.28	0.56	0.43
C4	.0180	.0104	.0050	0.48	0.28	0.58
R6 C1	.0216	.0026	.0150	5.77	0.69	0.12
C2	.0216	.0052	.0100	1.92	0.46	0.24
C3	.0216	.0078	.0050	0.64	0.23	0.36
R7 C1	.0252	.0026	.0100	3.85	0.40	0.10
C2	.0252	.0052	.0050	0.96	0.20	0.20
R8 C1	.0288	.0026	.0050	1.92	0.17	0.09

[1] These ratio values are based on the supposition that the salts are completely ionized, so that all the Mg, etc., in the solution is regarded as being in the form of Mg, etc., ions. This is, of course, not strictly true, but as the ions are absorbed by the plant more ions should be formed, so that eventually all of these atoms should be available as ions.

A continuous record of the temperature changes during the period of this series was secured by means of a thermograph. The highest temperature recorded was 34° C. (on May 22 and 25) and the lowest was 9° C.

(on May 18 and 20). A cylindrical porous-cup atmometer, which was used to indicate the variations in the evaporating power of the air during the period in question, gave a daily mean water loss of 7.6 c.c., a maximum for a 24-hour period of 13.6 c.c. (on June 5), and a minimum of 1.4 c.c. (on May 30), with a total loss of 182 c.c. for the entire period of 24 days. These readings are all corrected to the Livingston standard cylindrical cup (21) by multiplying the actual readings by the coefficient furnished with the instrument.

In the following sections will be found a discussion of the physiological effects upon the wheat plants, produced by the various solutions, with their different salt-proportions, and a comparison of these results from sand cultures with those obtained by Shive from his corresponding water cultures, and also with Shive's results from his sub-optimal series, in which the total osmotic concentration was 0.1 atmosphere. The behavior of the plants in the different cultures will be compared with reference to the dry weights of tops and of roots and with respect to the relative amounts of water transpired during the growth period.

II. Dry Weights

(a) Method Employed in Harvesting

At the end of the growth period the wax seal was removed from the surface of the sand and the contents of the culture pot were carefully transferred to a coarse sieve having approximately 10 meshes to the inch. By means of a gentle stream of water the sand was then washed down through the sieve, leaving the plants and roots behind. Tho tops of the plants were severed from the roots just above the remnant of the seed and then dried in an electric oven at 80° C. for a period of 24 hours, after which they were dried to constant weight at an oven temperature of approximately 102° C. Since it was impossible to wash the sand entirely free from the roots, a different method of procedure was necessary in order to obtain the dry weight of these subterranean parts. Without attempting to remove the last traces of sand, the roots were transferred from the sieve to a piece of paper and allowed to attain an air-dry condition, after which they were dried in the oven, just as in the case of the tops, until the oven-dry weights were obtained. These included, for each lot of roots, the weight of the adhering sand as well as the weight of the roots themselves. To correct this error the oven-dried roots were ignited in porcelain crucibles until all the organic matter had been destroyed. The loss on ignition of the samples was recorded as representing the approximate dry weight of the roots, upon the assumption that the sand adhering to the roots was all non-combustible and therefore suffered no loss in the ignition process. For practical purposes the small amount of ash resulting from the ignition of the root tissues may be neglected, especially since the relative weights would be affected only by the difference between the weights of the ash from the various individual cultures.

(b) Presentation of Data

In Table VI are presented the transpiration data for the individual cultures, together with the dry weights of tops and of roots. The transpiration data include (1) the actual water loss, in cubic centimeters, of

TABLE VI

TRANSPIRATION RECORD AND DRY WEIGHTS OF TOPS AND OF ROOTS FOR WHEAT GROWN 24 DAYS IN SAND CULTURES, SUPPLIED WITH THREE-SALT SOLUTIONS, ALL HAVING A TOTAL OSMOTIC CONCENTRATION OF 1.75 ATMOSPHERES, BUT DIFFERING FROM EACH OTHER IN THE PROPORTION OF THE THREE SALTS EMPLOYED

Culture Number	Transpiration (6 Plants)		Dry Weight of Tops[1] (6 Plants)		Dry Weight of Roots[1] (6 Plants)	
	Total Water Loss	Relative to R1 C1 as Unity	Absolute	Relative to R1 C1 as Unity	Absolute	Relative to R1 C1 as Unity
	gm.		gm.		gm.	
R1 C1	175.3	1.00	0.6412	1.00L	.1180	1.00L
C2	245.7	1.41	0.9504	1.48	.2118	1.79
C3	273.3	1.56	1.0723	1.67	.1672	1.41L
C4	271.2	1.55	1.1276	1.75H	.1936	1.63L
C5	316.2	1.80	1.0612	1.65	.2171	1.83
C6	321.6	1.83	1.1882	1.85H	.2174	1.84
C7	341.2	1.95	1.2181	1.90H	.2318	1.95
C8	339.9	1.94	1.2811	2.00H	.2168	1.83
R2 C1	205.8	1.17	0.6285	0.95L	.1844	1.55L
C2	248.3	1.42	0.8474	1.32	.2134	1.88
C3	303.8	1.73	1.0445	1.68	.2210	1.88
C4	353.7	2.00	1.2770	2.00H	.2652	2.24H
C5	339.5	1.94	1.1428	1.78H	.3166	2.67H
C6	321.8	1.83	1.1420	1.78H	.2597	2.19
C7	391.0	**2.23H**	1.4660	**2.29H**	.3333	**2.81H**
R3 C1	236.3	1.34	0.7080	1.11L	.2828	2.38H
C2	306.0	1.75	1.0358	1.62	.2969	2.50H
C3	263.8	1.50	0.9072	1.43	.2471	2.08
C4	312.0	1.78	1.0140	1.58	.2642	2.23H
C5	314.8	1.79	1.0810	1.71	.2786	2.35H
C6	336.1	1.92	1.0972	1.73H	.2077	1.75
R4 C1	192.2	1.09	0.5201	0.86L	.2531	2.13
C2	308.3	1.75	1.0330	1.62	.2969	2.50H
C3	260.6	1.50	0.8310	1.30L	.2316	1.95
C4	327.9	1.87	1.1033	1.72	.2630	2.22
C5	302.5	1.72	0.9848	1.54	.2440	2.06
R5 C1	237.6	1.35	0.6822	1.07L	.2440	2.06
C2	251.4	1.44	0.7790	1.23L	.1786	1.50L
C3	251.6	1.44	0.7763	1.21L	.1794	1.51L
C4	289.4	1.65	0.8912	1.39	.1549	1.31L
R6 C1	261.3	1.50	0.8489	1.32	.1527	1.30L
C2	306.7	1.75	0.9151	1.43	.1730	1.46L
C3	358.1	2.05	0.9460	1.48	.2598	2.19
R7 C1	208.6	1.19	0.6285	0.95L	.2044	1.72
C2	286.8	1.65	0.9540	1.50	.2266	1.91
R8 C1	277.6	1.60	0.8466	1.32	.2669	2.25H

[1]The best nine cultures are marked H, while the poorest nine are marked L.

each individual culture for the entire growth period and (2) these same quantities expressed as relative to the loss from culture R1 C1, taken as unity. In the dry weight columns are recorded (1) the absolute dry

weights, in grams, of both tops and roots separately and (2) the weights of tops and of roots relative to those of culture R1 C1, taken as unity. The maximum transpiration and the highest yields of tops and roots are here indicated by black-face type. A discussion of the transpiration data will be presented under a separate heading, following the discussion devoted to a comparison of the results obtained in the sand cultures with those secured in solution cultures.

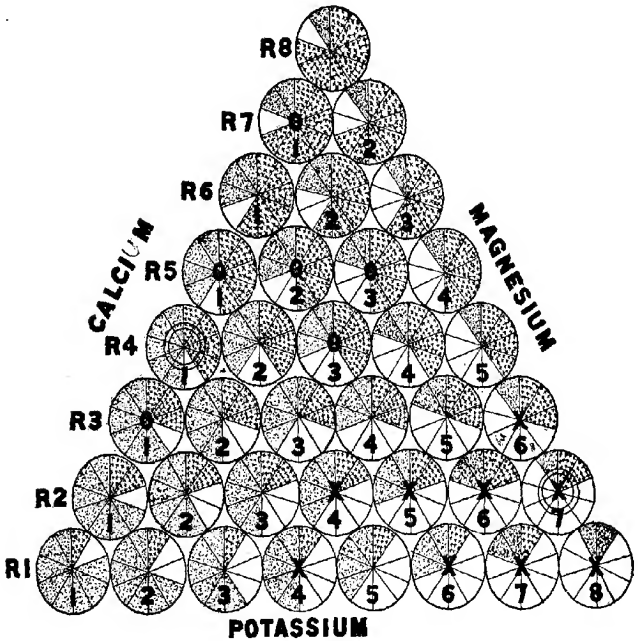

Fig. 2.—Triangular diagram showing the arrangement of the sand cultures with respect to the partial concentrations of the three salts employed. Unshaded segments represent the proportions of $Ca(NO_3)_2$: stippled segments the Mg SO_4: and the segments shaded with crosses the KH_2PO_4. The best nine cultures are marked X, while the poorest nine are marked by O.

In order to study better the relative growth rates, the entire series of 36 cultures are divided into three groups, (1) a lower one-fourth composed of the 9 cultures giving the lowest yields (either tops or roots), (2) an upper one-fourth composed of the 9 cultures giving the highest yield values, and (3) a medium one-half which comprised the remaining cultures. To facilitate the comparison, the solution cultures (38) were treated in exactly the same manner. In Tables VI and VII the relative

yields are marked with an L if they lie in the low yield group and with an H if they lie within the high yield group. The cultures with high and with low values for weights of tops are shown on the diagram of figure 2. These, and similar groups of cultures, are to be found on the diagrams of figures 3 and 5, and will be referred to, always, as the *best* nine and the *poorest* nine.

III. A Comparison of the Results from Sand Cultures with those from Solution Cultures

(a) Dry Weight of Tops

As has been mentioned, the results here brought forward were secured by using seed from the same lot as that from which Shive's seed was selected, and it thus seems desirable to compare the growths secured with these sand cultures with those obtained by Shive with solution cultures.

The second and third columns of Table VII present the relative dry weights of tops and of roots for the various sand cultures here employed, which were all supplied with nutrient solutions of the same total osmotic concentration, but of different salt-proportions, as already described. In columns 4 and 5 of this table are given the relative dry weights of tops and of roots secured by Shive in his sub-optimal cultures, all these solutions having a total osmotic concentration of 0.1 atmosphere. In the two columns at the right of the table are given the relative dry weights of tops and of roots for Shive's optimal series, with solutions having the same total osmotic concentration (1.75 atmospheres) as those employed in the sand cultures of the present study. The *actual* dry weights, in grams, of culture R1 C1 are given in parentheses directly below the relative weight values. The actual dry weight of any culture may be obtained by multiplying the relative weight by the actual weight of culture R1 C1 as given in the same column. Shive's supra-optimal series of cultures, with total concentration of 4.0 atmospheres, is not here considered.

To facilitate a general comparison of these three sets of cultures, the relative yields of tops are graphically shown in the triangular diagrams of figure 3, where A represents the sand cultures and B and C represent Shive's sub-optimum and optimum series, respectively. The areas of high yields are here indicated by crosses and those of low yields are shown by small circles, as was done by Shive. In each diagram the culture giving the highest yield is indicated by a large X.

It is readily apparent that there is a marked similarity, with respect to the location of the area of the poorest growth of tops, between the diagram for the sand cultures (1.75 atmospheres, total concentration, fig. 3, A) and that representing Shive's sub-optimal series (0.1 atmosphere, total concentration, fig 3, B). In fact, all three of the diagrams show a mark-

ed similarity in this respect. The culture giving the highest yield of tops in the sand series is R2 C7, which is characterized by having 2 tenths of

TABLE VII

COMPARISON OF THE RELATIVE DRY WEIGHTS OF TOPS AND OF ROOTS OF WHEAT GROWN IN SAND CULTURES WITH CORRESPONDING DATA FOR WHEAT GROWN IN SOLUTION CULTURES

Culture Number	Sand Cultures (McCall) Total Concentration of Solution 1.75 atm.		Sub-optimal and Optimal Solution Cultures (Shive, 1915)			
			Total Concentration 0.1 atm.		Total Concentration 1.75 atm.	
	Relative Dry Weight[1]		Relative Dry Weight		Relative Dry Weight	
	Tops	Roots	Tops	Roots	Tops	Roots
R1 C1	1.00L	1.00L	1.00L	1.00	1.00L	1.00
	(0.6412)	(0.1186)	(0.2601)	(0.1036)	(0.4104)	(0.1058)
C2	1.48	1.79	1.22	1.05	1.19	1.11H
C3	1.67	1.41L	1.25	0.99	1.20	0.93
C4	1.75H	1.63L	1.30	0.91	1.17L	1.07H
C5	1.65	1.83	1.24	0.86L	1.26	0.99
C6	1.85H	1.84	1.38	0.98	1.16L	1.03
C7	1.90H	1.95	1.25	0.78L	1.11L	1.01
C8	2.00H	1.84	1.23	0.80L	1.17	0.95
R2 C1	0.95L	1.55L	1.03L	1.19	1.03L	0.96
C2	1.32	1.88	1.20L	1.04	1.14L	1.05H
C3	1.68	1.88	1.39	0.93	1.25	0.93L
C4	2.00H	2.24H	1.48H	0.94	1.27H	0.95
C5	1.78H	2.67H	1.40	0.77L	1.18	0.90L
C6	1.78H	2.19	1.43H	0.76L	1.22	0.98
C7	2.29H	2.81H	1.39	0.80L	1.23	1.04
R3 C1	1.11L	2.38H	1.11L	1.39H	1.15L	1.02
C2	1.16	2.50H	1.28	1.19H	1.24	1.07H
C3	1.43	2.08	1.42H	1.00	1.36H	1.07H
C4	1.58	2.23H	1.57H	0.94	1.28H	0.95
C5	1.71	2.35H	1.52H	0.82L	1.25	0.92L
C6	1.73H	1.75	1.35	0.91	1.27H	0.93L
R4 C1	0.86L	2.13	1.00L	1.31H	1.12L	1.04
C2	1.62	2.50H	1.21	1.22H	1.28H	1.10H
C3	1.30L	1.95	1.41	1.06	1.26	0.91L
C4	1.72	2.22	1.57H	0.89	1.27	0.91L
C5	1.54	2.06	1.65H	0.89L	1.30H	1.04
R5 C1	1.07L	2.06	1.09L	1.30H	1.19	1.07H
C2	1.23L	1.50L	1.47H	1.18	1.39H	1.08H
C3	1.21L	1.51L	1.41	0.90	1.24	0.91L
C4	1.39	1.31L	1.32	0.72L	1.28H	1.03
R6 C1	1.32	1.30L	1.10L	1.31H	1.17	1.06H
C2	1.43	1.46L	1.29	1.07	1.19	0.91L
C3	1.48	2.19	1.44H	1.02	1.21	0.87L
R7 C1	0.95L	1.72	1.11L	1.36H	1.16L	1.03
C2	1.50	1.91	1.29	1.28H	1.31H	0.98
R8 C1	1.32	2.25H	1.10L	1.35H	....	....
Check[2]	0.52	1.78	....	....	....	....

[1] The dry weight of culture R1 C1 is always taken as unity and the other weights are expressed in terms of this value. The actual dry weight of culture R1 C1 is given in parentheses, in grams. The best nine cultures are marked H, while the poorest are marked L.

[2] Check received only distilled water.

its total osmotic concentration derived from mono-potassium phosphate, 7 tenths from calcium nitrate and 1 tenth from magnesium sulphate. This

culture gave a yield of dry tops 129 per cent greater than that of R1 C1. The culture giving the highest yield of tops in Shive's sub-optimal solution cultures was R4 C5, its yield being 65 per cent higher than that from culture R1 C1 in the same series. This culture solution is characterized

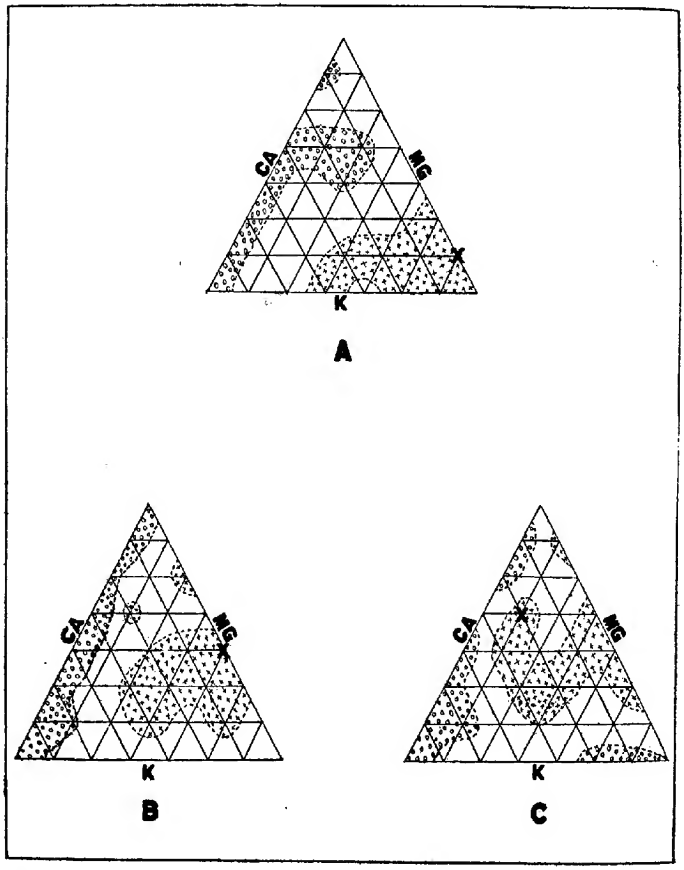

Fig. 3.—Triangular diagrams showing areas of high and of low yield of tops. A, sand cultures; B, Shive's sub-optimal, and C, Shive's optimal solution cultures.

by having 4 tenths of its total osmotic concentration derived from monopotassium phosphate, 5 tenths from calcium nitrate and 1 tenth from magnesium sulphate. In Shive's optimal series (1.75 atmospheres) the highest dry weight of tops was obtained from culture R5 C2, and this

yield was 39 per cent higher than that from culture R1 C1 in the same series. This solution is characterized by having 5 tenths of its total osmotic concentration due to mono-potassium phosphate, 2 tenths to calcium nitrate, and 3 tenths to magnesium sulphate. These data are summarized in Table VIII.

TABLE VIII

OSMOTIC PROPORTIONS OF THE THREE-SALT SOLUTIONS GIVING THE BEST GROWTH OF TOPS IN THE SAND CULTURE SERIES (McCALL) AND IN THE SUB-OPTIMAL AND OPTIMAL SOLUTION SERIES (SHIVE)

Series	Relative Dry Weight of Tops	Total Concentration atm.	Osmotic Proportions, in Tenths, of of Total Concentration		
			KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
Sand Cultures (McCall)	2.29	1.75	2	7	1
Sub-optimal (Shive) ..	1.65	0.10	4	5	1
Optimal (Shive)	1.39	1.75	5	2	3

A comparison of the best sand culture (total concentration 1.75 atmospheres) with the best solution culture of the sub-optimal series (total concentration 0.1 of an atmosphere) brings out the fact that the osmotic concentration ratio of magnesium sulphate to mono-potassium phosphate plus calcium nitrate is the same for both cultures, namely 1:9. The ratio of magnesium sulphate to calcium nitrate is 1:7 for the sand culture and 1:5 for the solution culture. The greatest difference between the relative proportions of the salts employed is shown by the ratio of calcium nitrate to mono-potassium phosphate, this ratio being 7:2 for the sand and 5:4 for the solution culture.

A comparison of the best sand culture with the solution culture giving the best growth of tops in Shive's optimal series, having the same total osmotic concentration (1.75 atm.) brings out some surprising results. In these two cultures there is a marked difference in salt proportions. Shive's best solution in his optimal series is characterized by a value of 3:7 for the osmotic ratio of magnesium sulphate to calcium nitrate plus mono-potassium phosphate; while in the best culture of the sand series and the best in the sub-optimal solution series this ratio is 1:9, as has already been stated. The most striking difference between the best culture of the sand series and the best of Shive's optimal water-culture series is found, however, in the relation of magnesium to calcium. In the best solution of Shive's series the osmotic ratio of magnesium sulphate to calcium nitrate is 3:2, while for the best culture of the sand series this ratio is 1:7. The osmotic ratio of the calcium salt to the potassium salt is also markedly different in these two cases, being 2:5 for the best solution culture and 7:2 for the best sand culture.

While it appears to be impossible to draw any definite conclusions from a detailed study of the characteristics of the solutions that produced

the best growth of tops in the three series it is important to note (1) that the results secured in the sub-optimal solution series are more nearly like those from the sand series than are the results obtained from the optimal solution series with the same total osmotic concentration; (2) that there is a marked difference between the solutions producing the best growth of tops in sand and those giving the best growth of tops in solution cultures, with respect to the osmotic proportions of the three salts employed. Attention is called to the fact that the improvement in growth of tops as we proceed to the right from the left margin of the triangle is very much more marked in the sand than in the solution series. This is brought out in a striking manner by the graphs of figure 4, which show the variations in the yield of tops of the individual cultures in the sand series and in Shive's optimal and sub-optimal solution series.

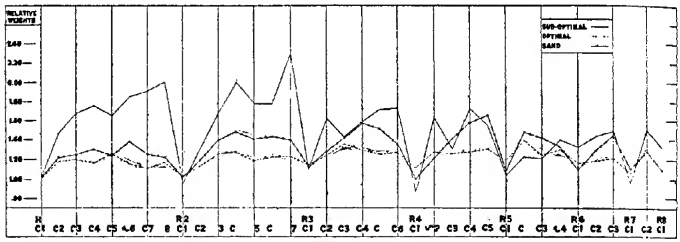

Fig. 4.—Relative dry weights of cultures grown in sand and in solution cultures of sub-optimal and of optimal total concentrations.

The most striking feature of these graphs is the regularity with which the one for the sand series of cultures intersects those for the two solution series. It will be seen that this intersection occurs always at culture No. 1 of each row, as represented on the triangular diagram, with the exception of R6 C1 and of R8 C1. A possible explanation of the phenomenon thus indicated is offered by the known selective adsorptive property of sand and other finely divided substances.

As early as 1866, Frank (11), studied the retention of potassium chloride by the soil, finding that soil has the power of absorbing, or removing from solution, considerable amounts of this salt. Subsequent investigations have shown that this power to remove salts from solution is also possessed by other finely divided substances that are chemically inert, such as charcoal and pulverized silica. More recent work has not only confirmed these early observations, but has brought out the fact that finely divided substances may exercise a selective action with respect to the solution with which they are brought into contact. In some cases the effect of this selective

action is to remove one ion of the salt more rapidly than the other, leaving the solution alkaline or acid, depending upon which ion is removed to the greater extent.[1] It has also been found that, in certain cases, the selective adsorption increases in amount with the concentration of the solution, up to a certain maximum, and then remains constant with still further increase in concentration.

To employ these considerations in an attempt to explain the physiological phenomena in question, it may be supposed that the poor growth of all of the C1 cultures, whether in sand or solution, is due to a deficiency of calcium nitrate together with the accompanying excess of magnesium sulphate, thus leaving out of account, for the present, row 6 and the single culture at the apex of the diagram. In the solution cultures, as we pass from C1 to C2, in each row, the proportion of calcium nitrate to magnesium sulphate becomes slightly more favorable, giving rise to a slight increase in the dry weight from C2 as compared with C1. It may also be supposed that the partial concentration of the magnesium salt is here the factor limiting the growth of the plants and that this salt antagonizes the NO_3 radical, thus preventing the latter from exerting an accelerating influence on the growth rate. In the sand cultures it is possible that the NO_3 radical is not appreciably adsorbed by the sand, but that a part of the magnesium present in the system is so adsorbed, thus being prevented from active participation in the physiological processes of the plants.[2]

To go farther with this hypothesis, it may be supposed that this adsorption of magnesium is not sufficient, in the left cultures of the diagram, to allow the NO_3 ions to assert themselves by accelerating the growth of the plants. As we proceed toward the right, on the triangular diagram, the partial concentration of magnesium sulphate in the original solution decreases by increments of 1 tenth of the total concentration, from each culture to the next. Now, the magnesium sulphate actually free to affect the plants of the sand cultures is the amount of this salt in the original solution minus the amount that has been adsorbed by the sand, and it may thus be that the very marked progressive improvement in growth as we proceed from left to right across the diagram is due to a parallel increase in the partial concentration of the calcium nitrate accompanied by a corresponding decrease in the magnesium sulphate. This alteration in the salt-proportions (or ion-proportions) of the unadsorbed solution brought about by the selective adsorption of magnesium sulphate by the solid medium, may give rise to a better physiological balance than that which characterizes the unmodified solution.

[1] See in this connection: Gore, G. (12); Briggs, J. L. (3); Cameron, F. C., and Bell, J. M. (6); Parker, E. G. (29); Williams, A. M. (45); and McCall, A. G. (26).

[2] While no direct evidence bearing upon this point can be produced, it may be mentioned that Parker (29, p. 188) found that sodium nitrate in certain partial concentrations increased the adsorption, by the soil, of potassium chloride out of the same solution.

(b) Dry Weight of Roots

The dry weights of roots are given in Table VII, in connection with the dry weights of tops. The graph presented in figure 6 shows the variation in dry weight of roots between the individual cultures. The marked characteristic of this diagram is the manner in which the graph makes a gradual rise to near the middle of the series and then gradually declines until near the end, where a slight rise again occurs.

The discussion of these data can best be presented by referring to the triangular diagram of figure 5A, in which the area of high relative values is indicated by crosses and that of low values is marked by circles. The relative dry weights of roots have a total range from 1.00 (culture R1 C1) to a maximum of 2.81 (culture R2 C7). A comparison of this diagram with figure 3A, giving the dry weights of tops, brings out the interesting fact that the culture showing the best growth of roots (culture R2 C7) is also the one that gave the highest yield of tops. This culture gave 129 per cent greater yield of tops and 181 per cent greater yield of roots than did culture R1 C1. No such correlation is apparent, however, between the culture giving the poorest yield of tops and the one showing the poorest root development.

Considering, now, the areas of high and of low root yields, it will be seen that an area of high yields (2.22 to 2.81) extends nearly across the entire width of the triangle, in a regular belt beginning at row 2 on the right and passing obliquely upward to the center of the triangle (where a slight break occurs) thence to the right margin at row 3. In a similar manner an area of low yields (1.30 to 1.51) extends across the entire width of the triangle, occupying a position above the area of high yields and being confined to rows 5 and 6. Two small areas of low yields are shown at the lower margin of the diagram, each of which includes two cultures.

The solution giving the best growth of roots, as in the case of tops, is characterized by having 2 tenths of its total osmotic concentration derived from mono-potassium phosphate, 7 tenths from calcium nitrate and the remaining 1 tenth from magnesium sulphate.

The culture giving the poorest growth of roots is culture R1 C1, which lies outside of the main area of low root yields. This culture has 1 tenth of its total osmotic concentration due to mono-potassium phosphate, 1 tenth to calcium nitrate, and the remaining 8 tenths to magnesium sulphate.

A comparison of this diagram with the corresponding diagrams (figure 5, B and C) of Shive's solution cultures serves to bring out the fact that the areas of highest and of lowest yields of roots extend in narrow strips across the triangular diagram for the sand cultures in a direction almost at right angles to the direction taken by the corresponding areas on the diagrams representing the solution cultures with total concentra-

tion of 0.1 and 1.75 atmospheres. A comparison of the root yields obtained from the sand cultures with those obtained from the solutions of the same total concentration as was used in the sand series (1.75 atm.) and also with those obtained from solutions having a concentration of 0.1 atmosphere fails to reveal any further generalization that might be of interest or value in this connection.

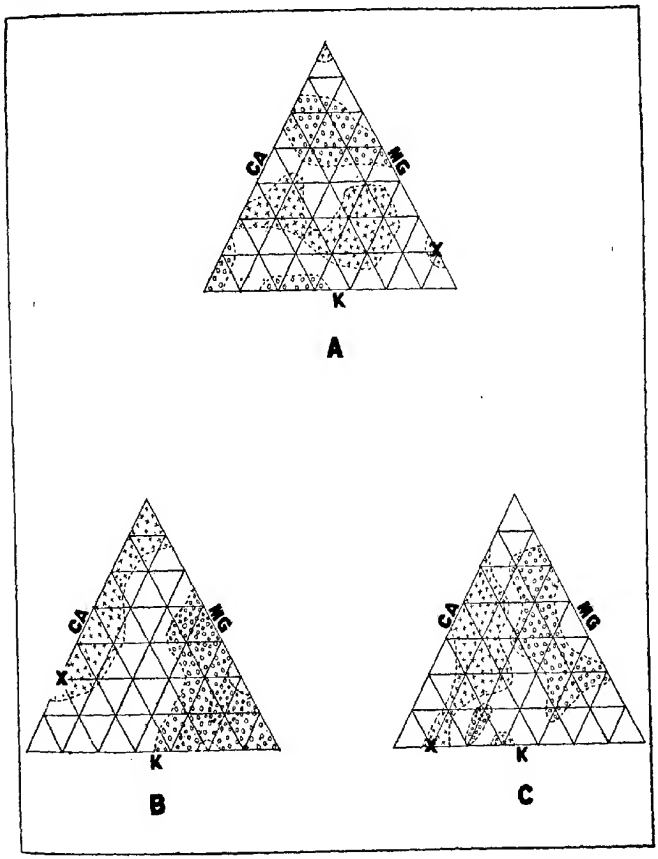

Fig. 5.—Triangular diagram showing areas of high and of low yield of roots. A, sand cultures; B, Shive's sub-optimal, and C, Shive's optimal solution cultures.

IV. Relation of Top Yields to Cation Ratio Values

Introduction. The dissociation of the three salts employed in these culture solutions gives rise to three kinds of cations (Ca, K and Mg) and three kinds of anions (NO_3, PO_4 and SO_4), if we assume a com-

plete ionization of the salts and disregard the H ions and the HPO_4 ions produced by the dissociation of mono-potassium phosphate. Since these chemical units also appear to function as units in the metabolic processes of the plant, and since absorption of any ion by the plant should lead to further production of that ion in the solution, until all of the molecules in question, originally present, had been separated into ions, it is of interest to study the relation between the ion ratios and the yield of tops, somewhat as was done by Shive in his study of solution cultures corresponding to the sand cultures here considered.

As the last mentioned writer has pointed out, one of these three-salt solutions may be defined by the three cation ratios, Mg/Ca, Mg/K and Ca/K, for the values of any other of the possible ion ratios may be found directly from these three. The cation ratio values alone will therefore be considered in this discussion.

It sould be noted at once that the ionic ratios may be very greatly modified by the presence of the sand, which, through its adsorptive power may not only markedly reduce total concentration of the original solution, but may also alter the original relative proportions of the component salts and ions. As has been emphasized above, the adsorptive action may fall more heavily on some of the ions than upon others, so that the unadsorbed solution remaining in the sand of the culture pots may be characterized by very different molecular and ionic ratios from those of the original solutions with which the pots were first saturated. It is highly probable that selective adsorption plays an important rôle in bringing about the physiological differences to be observed between the sand cultures of the present paper and the corresponding solution cultures of Shive's study.

Dry weight of tops. Range of cation ratios for the best nine cultures. Table IX presents the cation ratio values for each of the nine solutions (of the sand cultures and of the sub-optimal and optimal) that produced the highest yield of tops. The best nine cultures, in each case, are the ones marked H in Tables VI and VII. The cultures are here (Table IX) arranged in the descending order, on the basis of top yields, the one producing the highest yield in each series being placed at the head of the column. In the columns giving the cation ratios the minimum and the maximum values for these ratios are marked L and H, respectively. The total range in the magnitude of these ratio values is given at the bottom of the respective column.

It will be observed that the group of sand-culture solutions giving high yields of tops is characterized by a comparatively low range (2.16) in the Mg/Ca ratio value, extending from minimum of 0.24 to maximum of 2.40. This group of highest yielding cultures includes the lowest value of this ratio, but is restricted to the lower one-sixth of the total range of these ratio values. Both the Mg/K and the Ca/K ratio values show a

much larger range than does the Mg/Ca ratio. From a consideration of these data it may be concluded that in sand cultures, such as were here employed, we may expect to find good growth of tops associated with Mg/Ca ratio values between 0.24 and 2.40; a range of Mg/K ratio values from 0.46 to 6.94; and a range of Ca/K ratio values from 1.44 to 5.77. The culture (R2 C7) giving the largest yield of tops is characterized by a low Mg/Ca ratio (0.46), a low Mg/K ratio value (0.69) and an intermediate value (2.52) for the Ca/K ratio.

TABLE IX

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF TOPS OF THE BEST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Tops Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R2 C7	0.27	0.69	2.52	2.29
	R1 C8	0.24L	1.39	5.77H	2.00
	R2 C4	1.92	2.77	1.44	2.00
	R1 C7	0.55	2.78	5.04	1.90
	R1 C6	0.96	4.17	4.33	1.85
	R2 C5	1.15	2.05	1.80	1.78
	R2 C6	0.64	1.38	2.16	1.78
	R1 C4	2.40H	6.94H	2.88	1.75
	R3 C5	0.32	0.46L	1.44L	1.73
Range		2.16	6.48	4.33	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R4 C5	0.38L	0.35	0.90	1.65
	R3 C4	1.44	1.39	0.96	1.57
	R4 C4	0.96	0.69	0.72	1.57
	R3 C5	0.77	0.93	1.20	1.52
	R2 C4	1.92	2.77H	1.44	1.48
	R5 C2	2.88H	0.83	0.29L	1.47
	R6 C3	0.64	0.23L	0.36	1.44
	R2 C6	0.64	1.39	2.16H	1.43
	R3 C3	2.56	1.85	0.72	1.42
Range		2.50	2.54	1.87	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R5 C2	2.88	0.83	0.29	1.39
	R3 C3	2.56	1.85	0.72	1.36
	R7 C2	0.96	0.20L	0.20L	1.31
	R4 C5	0.33	0.35	0.90	1.30
	R4 C2	3.85H	1.39	0.36	1.28
	R5 C4	0.48	0.28	0.58	1.28
	R3 C4	1.44	1.39	0.96	1.28
	R3 C6	0.32L	0.46	1.44	1.27
	R2 C4	1.92	2.77H	1.44H	1.27
Range		3.53	2.57	1.24	

From a comparison of these ratio values with those obtained in the solution cultures, it will be seen that, with respect to the Mg/Ca ratio, there is substantial agreement, the range for the sand culture series being 2.16, that for Shive's solution culture series, with total osmotic concentration of 0.1 atm., 2.50, and for the solution cultures, with a total osmotic

concentration of 1.75 atm., the range is 3.53. The range of the Mg/K ratio value is much greater for the sand culture series than for the solution culture series, both of which are characterized by a medium low range for this ratio. The range of the Ca/K ratio value for the sand cultures is very wide, and includes all possible values except a few of the very lowest. The solution culture series, on the contrary, show low ranges of this ratio and include neither the high nor any of the extremely low ratio values.

From these data it appears that there is substantial agreement between the three series with respect to the range of the Mg/Ca ratio of the solutions employed, but that no such agreement is to be found with respect to the other two cation ratios here considered, hence we may conclude that for the three-salt solution here employed the ratio of magnesium to calcium is more important than the ratio of magnesium to potassium or of calcium to potassium, in determining the best yield of tops. Attention is here directed to the fact that the first mentioned ratio is very closely related to the lime-magnesia ratio, so much discussed, and for this reason it will receive attention in a special chapter.

Dry weight of tops. Range of cation ratios for the poorest nine cultures. Table X shows the cation ratio values for each of the nine solutions (both sand and solution culture series) that produced the lowest yield of tops. The poorest nine cultures, in each case, are the ones marked L in Tables VI and VII. The cultures are here (Table X) arranged in the ascending order, the one producing the lowest yield in each series being placed at the head of the column. As in the previous section (Table IX), the minimum and the maximum values for the ratios are marked L and H, and the total range in the magnitude of these ratio values is given at the bottom of the respective column.

It will be observed that the group of sand-culture solutions giving the lowest yield of tops is characterized by a very wide range in the Mg/Ca ratio values, which include all but the very lowest values. The Mg/K ratios cover practically the entire range of values, while the Ca/K ratio shows a very low range, which is confined to the low values. The poorest individual culture is characterized by a high (9.61) Mg/Ca ratio, a low (1.74) Mg/K ratio, and a very low (0.18) Ca/K ratio. From a consideration of these data, it may be concluded, that in sand cultures such as were here employed, we may expect to find poor growth of tops associated with a very low ratio of calcium to potassium. It appears, further, that the ratios of magnesium to potassium and of calcium to potassium are not important factors in bringing about a *poor* growth of tops.

From a comparison of these ratio values with those obtained in the solution cultures it will be seen that with respect to the range in the Mg/Ca and the Mg/K ratio values there is a very close agreement, the range being very wide for all three of the series here considered. With

respect to the range in the Ca/K ratios there is perfect agreement between the sand culture series (1.75 atm.) and the sub-optimal (0.1 atm.) solution culture series, the range in both cases being very narrow, but the optimal (1.75 atm.) solution culture series has a wide range for this ratio.

TABLE X

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF TOPS OF THE POOREST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Tops Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R4 C1	9.61	1.74	0.18	0.86
	R2 C1	13.46	4.86	0.36	0.95
	R7 C1	3.85	0.40L	0.10L	0.95
	R1 C1	15.40H	11.10	0.72H	1.00
	R5 C1	7.69	11.11H	0.14	1.07
	R3 C1	11.53	2.78	0.24	1.11
	R5 C3	1.28L	0.56	0.43	1.21
	R5 C2	2.88	0.83	0.29	1.23
	R4 C3	1.92	1.04	0.54	1.30
Range		14.12	10.70	0.62	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R1 C1	15.40H	11.10H	0.72H	1.00
	R4 C1	9.61	1.74	0.18	1.00
	R2 C1	13.46	4.86	0.36	1.03
	R5 C1	7.70	1.11	0.14	1.09
	R6 C1	5.77	0.69	0.12	1.10
	R8 C1	1.92L	0.18L	0.10L	1.10
	R3 C1	11.55	2.78	0.24	1.11
	R7 C1	3.85	0.40	0.10	1.11
	R2 C2	5.77	4.17	0.72	1.20
Range		13.48	10.92	0.62	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R1 C1	15.40H	11.10H	0.72	1.00
	R2 C1	13.46	4.86	0.36	1.03
	R1 C7	0.55	2.78	5.04H	1.11
	R4 C1	9.61	1.74	0.18	1.12
	R2 C2	5.77	4.17	0.72	1.14
	R3 C1	11.55	2.78	0.24	1.15
	R1 C6	0.96L	4.17	4.32	1.16
	R7 C1	3.85	0.40L	0.10L	1.16
	R1 C4	2.40	6.96	2.88	1.17
Range		14.44	10.70	4.94	

Dry weight of roots. Range of cation ratios for the best nine cultures. In Table XI are presented the cation ratio values for the best nine cultures (both sand and solution culture series) based on the dry weight of roots. As in case of the dry weight of tops, the best nine cultures in every case are the ones marked H in Tables VI and VII, the order of arrangement and the numbering of the high and low ratio values being the same as that followed in Table IX. It will be seen that the group of

sand culture solutions giving high yield of roots is characterized by a very wide range (11.26) for the Mg/Ca ratio values and medium low ranges (2.60 and 2.43, respectively) for the Mg/K and Ca/K ratios. The individual culture giving the largest weight of roots is characterized by very low values for both the Mg/Ca and the Mg/K cation ratios, and a medium value for the Ca/K ratio. The ratio ranges of the sand culture series

TABLE XI

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF ROOTS OF THE BEST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Roots Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R2 C7	0.27L	0.69	2.52H	2.81
	R2 C5	1.15	2.08	1.80	2.67
	R4 C2	3.85	1.39	0.36	2.50
	R3 C2	4.81	2.32	0.48	2.50
	R3 C1	11.53H	2.73H	0.24	2.38
	R3 C5	0.77	0.93	1.20	2.35
	R8 C1	1.92	0.18L	0.09L	2.25
	R2 C4	1.92	2.77	1.44	2.24
	R3 C4	1.44	1.39	0.96	2.23
Range		11.26	2.60	2.43	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R3 C1	11.55H	2.78H	0.24	1.39
	R7 C1	3.85	0.40	0.10	1.36
	R8 C1	1.92	0.18L	0.09L	1.35
	R4 C1	9.61	1.74	0.18	1.31
	R6 C1	5.77	0.69	0.12	1.31
	R5 C1	7.70	1.11	0.14	1.30
	R7 C2	0.96L	0.20	0.20	1.28
	R4 C2	3.85	1.39	0.36	1.22
	R3 C2	4.81	2.32	0.48H	1.19
Range		10.59	2.60	0.39	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R1 C2	6.74	9.72H	1.44	1.11
	R4 C2	3.85	1.39	0.36	1.10
	R5 C2	2.88	0.83	0.29	1.08
	R3 C2	4.81	2.32	0.48	1.07
	R1 C4	2.40L	6.95	2.88H	1.07
	R5 C1	7.70H	1.11	0.14	1.07
	R3 C3	2.56	1.85	0.72	1.07
	R6 C1	5.77	0.69L	0.12L	1.06
	R2 C2	5.77	4.17	0.72	1.05
Range		5.30	9.03	2.76	

show a substantial agreement with those of the solution culture series of sub-optimal (0.1 atm.) concentration with respect to the range in the Mg/Ca and Mg/K ratios, and a close agreement with the optimal solution (1.75 atm.) series with respect to the range in the Ca/K ratios. As was the case when groups of best cultures of the three series were compared on the basis of dry weight of tops, there is a much closer agreement with respect to the range in value of the Mg/Ca ratio than for the range

of Mg/K and Ca/K ratios. It appears, further, that the contact of the solution with the sand has not markedly changed the correspondence between the range of the Mg/Ca ratio and yield of tops and of roots.

Attention is called to the fact that the groups of best cultures on the basis of dry weight of tops were characterized throughout the three series by a comparatively narrow range in the value of the Mg/Ca ratio, while

TABLE XII

RANGE OF CATION RATIO VALUES AND RELATIVE DRY WEIGHTS OF ROOTS FOR THE POOREST NINE CULTURES OF WHEAT GROWN IN SAND AND IN SOLUTION CULTURES

	Culture Numbers	Cation Ratio Values			Yield of Roots Relative to that of Culture R1 C1
		Mg/Ca	Mg/K	Ca/K	
Sand cultures supplied with a solution having a concentration of 1.75 atm.	R1 C1	15.40H	11.10H	0.72	1.00
	R6 C1	0.48L	0.28L	0.58	1.30
	R5 C4	5.77	0.69	0.12L	1.31
	R1 C3	3.85	8.34	2.16	1.41
	R6 C2	1.92	0.46	0.24	1.46
	R5 C2	2.88	0.83	0.29	1.50
	R5 C3	1.28	0.56	0.43	1.51
	R2 C1	13.46	4.86	0.36	1.55
	R1 C4	2.40	6.94	2.88H	1.63
Range		14.92	10.82	2.76	
Solution cultures of sub-optimal concentration 0.1 atm. (Shive)	R5 C4	0.48	0.28L	0.58L	0.72
	R2 C6	0.64	1.29	2.16	0.76
	R2 C5	1.15	2.08	1.80	0.77
	R1 C7	0.55	2.78	5.04	0.78
	R2 C7	0.27	0.69	2.52	0.80
	R1 C8	0.24L	1.39	5.76H	0.80
	R3 C5	0.77	0.93	1.20	0.82
	R1 C5	1.54H	5.55H	3.60	0.86
	R4 C5	0.38	0.35	0.90	0.89
Range		1.30	5.27	5.18	
Solution cultures of optimal concentration 1.75 atm. (Shive)	R6 C3	0.64	0.23L	0.36L	0.87
	R2 C5	1.15	2.08	1.80H	0.90
	R4 C3	1.92	1.04	0.54	0.91
	R4 C4	0.96	0.69	0.72	0.91
	R5 C3	1.28	0.56	0.43	0.91
	R6 C2	1.92	0.46	0.24	0.91
	R3 C5	0.77	0.93	1.20	0.92
	R3 C6	0.32L	0.46	1.44	0.93
	R2 C3	3.21H	3.47H	1.08	0.93
Range		2.89	3.24	1.44	

the groups of best cultures on the dry weight of *roots* are characterized by a wide range in the same ratio.

Dry weight of roots. Range of cation ratios for the poorest nine cultures. Table XII shows the cation ratio values and their range for each series, the arrangement of the data being the same as that of the three tables directly preceding. The group of sand cultures shows a very wide

range in the Ca/K ratios. The individual culture showing the poorest growth is characterized by a very high (15.40) Mg/Ca ratio, a very high (11.10) Mg/K ratio, and a low (0.72) Ca/K ratio value. A comparison of the sand and the solution cultures fails to bring out any correlation with respect to the ranges in the cation ratio values as set forth in this table.

V. Water-Requirements

(a) Transpiration Data

Throughout the entire growth period of these cultures the pots were weighed, and the transpiration loss was recorded, at the end of each 3-day interval. The total water loss for each culture was then determined by summing the losses thus recorded for the entire period. The transpiration data for the entire series has been presented in Table VI in connection with the dry weights of tops and roots. In that table the water-losses have been expressed in terms of the loss from culture R1 C1. To bring out the close agreement between the relative water-loss and dry weight of tops, these data have been plotted as shown in the graphs presented in

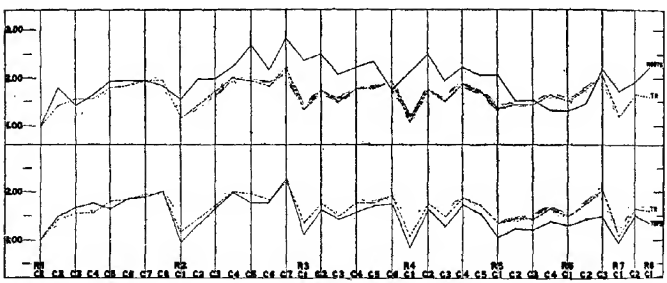

Fig. 6.—Relative transpiration and dry weight of tops and of roots of wheat grown in sand cultures for a period of 24 days.

figure 6. The abcissas are taken to represent the different cultures and the ordinates to indicate the relative dry weights and the transpiration losses relative to the loss from R1 C1 taken as unity. The broken line represents the variation in the relative dry weights of the individual cultures, while the solid line shows the variation in the water-loss from the same cultures.

The data presented in Table VI, and shown by the graphs of figure 6, appear to support the conclusion of Whitney and Cameron (44) and of other workers (20, 22) in the United States Bureau of Soils, to the effect that the total transpirational loss from a plant culture is approximately proportional to the growth made by the plants during the period of time considered. However, as has been pointed out by Livingston (19), this

generalization may be considered as approximately true only for plants of the same species and of the same age, grown with the same aerial environment but in different solutions. This writer concludes that, under the conditions of his experiments the amount of water lost by transpiration is roughly proportional to the extent of the leaf surface of the plant, which, in turn, is related to the size and, therefore, roughly, to the dry weight. Shive (38, p. 375) has emphasized the point that this relation can hold only where the transpiration loss is determined almost entirely by the size of the plant (area of its surface), and not by internal physiological conditions.

(b) Water-Requirement per Gram of Dry Tops

Introductory considerations. The ratio between the amount of water lost by transpiration during a period, and the dry weight of plants produced in the same time, is a convenient term by which to express the water requirements of the plants, since such a ratio is the quantitative expression of the number of grams of transpirational water required to produce a single gram of dry substance.

An excellent review of the early literature upon this subject has been published recently by the United States Department of Agriculture (5) and need not be reviewed in this paper. However, attention should be called to the fact that the results obtained by Sorauer and by Heinrich in controlled solution cultures are in agreement with the recent work of Shive (38, p. 378) with respect to the effect of the total concentration upon the water-requirements of the plants. The results brought forward by these investigators appear to lead to the general conclusion that the higher the total concentration of the nutrient medium, the lower is the ratio between the amount of water transpired and the dry yield of the plants. However, this generalization can hold only within a certain range of concentrations, since it is obvious that as the concentration is increased a point must be reached finally where no growth is possible. The present sand culture series offers an opportunity to study the variation in the water-requirements of these wheat plants when grown in solutions of the same total concentration (approximately optimal), but with a wide variation in the relative partial concentrations of the three salts employed.

Presentation of data. In columns 2, 3 and 4 of Table XIII the absolute transpiration ratios for tops, for roots, and for the entire plant, including both tops and roots, are given. These values were obtained by dividing the total water-loss from the individual cultures by the corresponding dry weight yields. In the last column of the table are also presented the ratios of top yield to root yield.

The data of Table XIII are shown graphically in figure 7, in which the upper graph represents the variation among the individual cultures

in the ratio of dry weight of tops to dry weight of roots, while the lower graphs represent the corresponding variations in the water-requirements for roots, for tops, and for entire plants including both tops and roots.

TABLE XIII

WATER-REQUIREMENTS FOR TOPS, ROOTS, AND ENTIRE PLANTS, AND THE RATIO OF TOPS TO ROOTS: WHEAT GROWN FOR 24 DAYS IN SAND CULTURES AND SUPPLIED WITH A THREE-SALT SOLUTION HAVING A TOTAL OSMOTIC CONCENTRATION OF 1.75 ATMOSPHERES

Culture Numbers	Water-Requirements[1]			Ratio[2] of Tops to Roots
	Tops	Roots	Entire Plant	
R1 C1	237L	1486	231	5.44
C2	258L	1160	211	4.49
C3	255L	1635	220	6.41
C4	240L	1401	205	5.82
C5	298	1475	247	4.89
C6	271L	1480	229	5.47
C7	280L	1472	235	5.25
C8	266L	1568	227	5.91
R2 C1	327H	1116	253	3.41
C2	293	1163	234	3.97
C3	291	1375	240	4.73
C4	277L	1334	229	4.82
C5	297	1072	233	3.61
C6	282	1239	230	4.40
C7	267L	1173	217	4.40
R3 C1	334H	828	238	2.50
C2	295	1031	230	3.94
C3	291	1068	228	3.67
C4	308	1181	244	3.84
C5	291	1130	232	3.95
C6	306	1618	258	5.28
R4 C1	370H	760	249	2.05
C2	298	1038	232	3.48
C3	326H	1125	245	3.59
C4	297	1247	240	4.20
C5	307	1240	246	4.04
R5 C1	348H	976	256	2.80
C2	323	1408	262	4.36
C3	324	1403	263	4.33
C4	325	1868	277	5.75
R6 C1	308	1711	261	5.56
C2	334H	1773	282	5.29
C3	378H	1380	297	3.64
R7 C1	332H	1021	250	3.07
C2	301	1266	243	4.21
R8 C1	328H	1040	249	3.14

[1] The water-requirement is determined by dividing the total water loss in grams by the dry weight value in grams.

[2] Obtained by dividing the dry weight of tops by the dry weight of roots.

The mean of the values for the water-requirements for tops is 307. It will be observed, in general, that the graph representing the ratio for tops (fig. 7) remains below the mean for the first twenty cultures, and that from this point forward the values equal or exceed the mean. From a study of the location of the cultures on the triangular diagram (fig. 8) it appears that a low water-requirement is associated with a low partial

osmotic concentration of mono-potassium phosphate. The location of each of the areas of high and of low water-requirements for tops is shown in figure 8, in which the area of high values is indicated by crosses and the area of low values is marked by small circles. As in case of dry weights, the shaded areas include the nine cultures showing the highest values and an equal number of cultures showing the lowest values. In Table XIII the nine cultures showing the highest water-requirements are marked H, while the nine showing the lowest water-requirements are marked L. The numerical values of the ratios for the different cultures are given on the diagram. A study of figure 9 with respect to water-

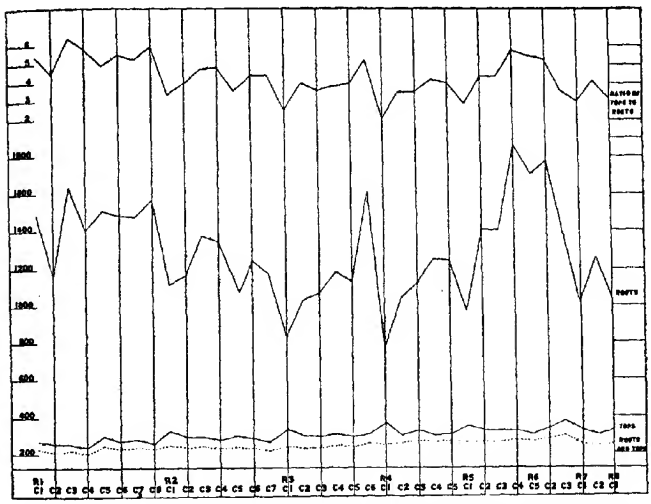

Fig. 7.—The water-requirement for the entire plant, for dry weight of tops and for dry weight of roots. The upper graph represents the variation in the ratios of tops to roots for the individual cultures.

requirements of the nine cultures giving the highest yield of tops and the nine cultures giving the lowest yields brings out the following facts. The average water-loss from the best nine cultures is 97 per cent greater than the water-loss from culture R1 C1, while the average yield of tops is only 89 per cent greater than that from culture R1 C1. The average water-loss from the poorest nine cultures is 27 per cent greater than that from culture R1 C1, while the average yield is 8 per cent greater than that from culture R1 C1. These observations are in accord with what might be expected from *a priori* grounds and from previous studies of water-requirement, namely, that favorable conditions for plant growth are generally associated with low water-requirements and that unfavorable conditions are concomitant with an increased water-requirement.

(c) Water-Requirement per gram of Dry Roots

The mean of the values for the water-requirements for dry weight of roots is 1285. It will be observed from the graph for this value in figure 7 that there is a region of high values near each end, with a distinct region of low values between. From a comparison of this graph with the graph representing the ratio of top yields to root yields it will be seen that the two lines follow each other quite closely. In every instance a

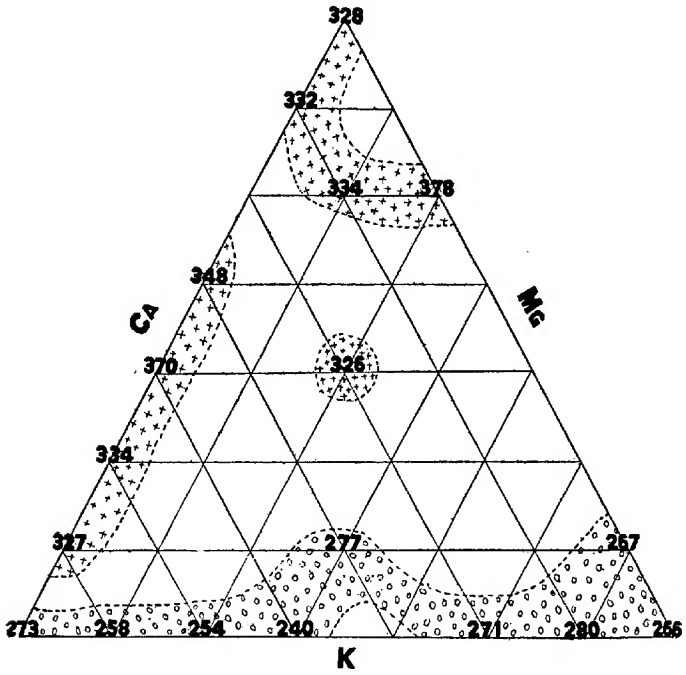

Fig. 8.—Areas of high and of low water-requirement values. The figures give the absolute values for 18 selected cultures.

high water-requirement corresponds to a high ratio of tops to roots and a low water-requirement corresponds to a low ratio of tops to roots. This is in harmony with what might be expected, since a high ratio of tops to roots suggests a large evaporating surface and a correspondingly high water-loss. As might be expected, the variation in the water-requirements is much greater for roots than for tops, the range for roots being from a minimum value of 760 (culture R1 C1) to a maximum value of 1868 (culture R5 C4).

(d) Water-Requirement per gram of Entire Plant

In figure 7 the graph representing the water-requirement for the entire plant very closely parallels the graph giving the ratios for dry weight of tops, and both show the same tendency to rise as we proceed from left to right. However, there are absent from the former the very high points that are characteristic of the graph representing the water-requirement for dry weight of tops, and that characterize those cultures having low ratios of tops to roots.

From an inspection of the graphs presented in figure 7 it is apparent that for this series of sand cultures the range of the water-requirement values, between the different cultures throughout the series, is comparatively narrow, when the dry weight of the plant is taken as the basis from which this ratio is derived. The range for the entire series of 36 cultures is from a minimum of 205 (culture R1 C4) to a maximum of 297 (culture R6 C3).

VI. The effect of the Calcium-Magnesium Ratio upon the Growth Rate

(a) Introduction

As the result of his investigations concerning the physiological requirements of plants, Loew (23) has worked out an hypothesis concerning the functions of lime and magnesia in the nutrition and the growth of plants. Early in these studies he became greatly impressed by the reversal of the quantitative relations of the lime and magnesia in the straw and in the seeds of plants. A chemical study of the ash of many common field plants brought out the fact that in the grain the magnesia is greatly in excess of the lime, while in the straw the lime is in excess of the magnesia. For example, in case of wheat, the ash of the grain contains 3.5 per cent of CaO and 13.2 per cent of MgO, while the straw has 5.8 per cent of CaO and only 2.5 per cent of MgO (39). His study of these data and the results of his own investigations led him to formulate the theory concerning the relationship of calcium to the nucleo-proteids and the specific function of magnesium with respect to the translocation of phosphorus in the plant. The outstanding points of Loew's lime-magnesia ratio hypothesis are as follows:

(1) When lime occurs in the growth medium greatly in excess of magnesia, or when magnesia occurs greatly in excess of lime, an injurious effect upon the plant is to be noted.

(2) Either of these elements tends to neutralize the harmful effects of the other.

The outstanding conclusions which Loew has drawn from his own and other experimental data are as follows:

(1) Different plants require, for their best growth, slightly different proportions of lime and magnesia.[1]

[1] In practically all of the literature the results are discussed in terms of the ratio of CaO to MgO rather than the ratio of CaO to Mg, hence the use of the terms "lime" and "magnesia" in this paper.

(2) The optimum ratio of lime to magnesia for oats is 1:1, for barley 2:1, and for buckwheat 1:3.

Upon the basis of various physiological investigations the writer just mentioned recommended that the amount of available lime and magnesia should be determined in agricultural soils in order to ascertain the proper lime-magnesia ratio. In a later publication Loew (24) discusses the physiological functions of calcium and magnesium in the plant. According to this writer, the presence of lime is necessary for the formation of certain calcium compounds required in the nuclei and in the chlorophyll bodies of the plant, and that magnesia assists in the assimilation of phosphorus, since magnesium phosphate can give up its phosphoric acid more easily than any other phosphate that occurs in the plant sap. The element calcium, therefore, is fixed in the organized structure of the cells, while magnesium is movable and serves as a carrier of assimilable phosphoric acid, which rôle can be repeated several times. But, if lime is taken up in excess, the assimilation of phosphoric acid will be made more difficult, since the acid will combine with the lime and thus diminish the chances for the formation of magnesium phosphate. As a result of the excess of lime, therefore, the amount of available phosphoric acid may be reduced and the plant may experience a partial starvation. If, on the other hand, magnesia is in considerable excess, the calcium of the nuclei and chlorophyll bodies may be transformed in the presence of the soluble magnesium salts into magnesium compounds, by an exchange of bases. This transformation of the calcium nucleo-proteids changes their capacity for imbibition, much to the detriment of the plant. According to the law of mass action, however, this transformation may be prevented by the simultaneous presence of dissolved calcium salts.

Since the publication of the lime-magnesia hypothesis of Loew, many other investigators have published experimental results bearing on the question here brought up. In a recent paper, Lipman (17) gives a critical review of 64 papers, all of which report results bearing upon the hypothesis of the lime-magnesia ratio. Some of these results appear to support Loew's hypothesis, others are inconclusive, and a third group seem to cast very serious doubt upon the necessity for definite ratios of lime to magnesia for the best growth of plants. Of the 64 papers considered by Lipman, 24 claim to give positive evidence of the need for a definite lime-magnesia ratio in the soil; 26 report negative results; and the remainder make no claim to either positive or negative results. In much of this literature the experimental evidence is not conclusive nor convincing, since the results obtained may be explained without resort to the hypothesis of Loew. To quote from Lipman: "The toxic effects of magnesium in excess or when applied to the soil when not in excess are easily explained on the basis of its own specific physiological properties

and need no introduction of ratio considerations between itself and calcium to explain them. . . . . Calcium and magnesium are, of course, essential elements in the growth of plants. Their somewhat similar chemical nature does not give us leave, however, to place them in a similar physiological category, and indeed numerous investigations point to their total dissimilarity, so far as that is concerned; Loew's own investigations being perhaps the most important in support of that idea. But, in spite of that, it is inconceivable to any one who has in view the modern developments of plant physiology and physical chemistry, as well as the modern views on the soil and its solution, why it should be any more assumed that a proper ratio between calcium and magnesium is necessary than that a proper ratio between calcium and potassium, and between calcium and iron, and between calcium and other essential elements in the growth of plants, are necessary. . . . . The balance of the effects which are not accounted for by antagonism between the ions within the soil solution itself, may, so far as soils themselves are concerned, be just as easily explained on the basis of the effects of the applications on the physical conditions of the soil, on the chemical reactions following in the soil, on the bacterial flora, on the protozoan fauna, and on other fauna within the soil, as it can be, by introducing the rather far-fetched notion of the necessity of the lime-magnesia ratio."

It requires but a superficial examination of the literature to reveal the fact that, in a majority of the experiments heretofore carried out in this connection, no attempt has been made to differentiate between the purely physiological action of calcium and magnesium and what may be called their *environmental* effect. It has been found that, in certain soils where magnesia is in excess of lime, application of the latter substance has been very beneficial to the growth of crop plants. In such cases it is not necessary to suppose that the benefit comes from the correction of an unfavorable ratio between the calcium and magnesium. Indeed, it would seem just as plausible to assume that the good effect obtained is the result of the beneficial effect of the lime upon the bacterial flora of the soil, which effect may be so great as to obscure any physiological effects due to an improvement of the calcium-magnesium ratio, if such effects are actually present. In such a case, the addition of lime to the soil would be considered as affecting the bacterial environment of the plant, without reference to any direct effects produced upon the plant by the higher soil content of calcium.

The sand-culture method described in this paper seems to afford favorable conditions for studying the relation of the calcium-magnesium ratio to the growth of plants. The substratum of pure sand, in which the wheat plants of these studies were grown, furnishes physical conditions similar to those found in the open soil, but avoids many of the biological complications encountered with natural soils, which complications may

very readily obscure the physiological relations between the plant and the salts of its surrounding medium. Furthermore, the renewal of the nutrient solutions at the end of every 3-day period serves to maintain within the medium a fairly constant condition with respect to all of the salts contained in the solutions. If a definite value of the calcium-magnesium ratio is essential for the best development of the seedling, evidence of that fact should be expected from such a complete series of sand cultures as the one here employed, since one of the cation ratios (Mg/Ca) is very closely related to the much discussed lime-magnesia ratio. If, on the other hand, the physiological processes of the plant are dependent in this connection, upon the proper balancing of the solution as a whole, we should not expect to find here any definite correlation between the value of the calcium-magnesium ratio and the growth and development of the seedlings.

(b) Discussion of Data

Examination of the data presented by the diagram of figure 2, with respect to the osmotic proportions of calcium nitrate to magnesium sulphate in a number of selected cultures, brings out some points in this connection. In the culture giving the best growth of tops (R2 C7) the osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ is 7:1, while in the culture giving the poorest growth of tops (R4 C1) this ratio value is 1:5 (C.2:1.0). The average ratio of $Ca(NO_3)_2$ to $MgSO_4$ for the best nine cultures has a value of 2.4:1, while for the poorest nine cultures this value is 1:2.9. It is thus evident that good growth is associated with a high osmotic ratio of $Ca(NO_3)_2$ to $MgSO_4$ and that the poor cultures are characterized by a low value of this ratio. We are not justified, however, in drawing from these results, any definite conclusions with respect to the effect of the calcium-magnesium, as such, because of the fact that much of the superior growth, in the culture where $Ca(NO_3)_2$ is in excess, may be ascribed to the presence of a large amount of the NO_3 radical, which is known to be favorable to very vigorous vegetative growth.

It has long been known that most higher plants absorb the greater part of their nitrogenous food material in the nitrate form. Hellriegel and Wilfarth's (14) experiments with barley in sand cultures, where the nitrogen was supplied in the form of $Ca(NO_3)_2$, show a very marked increase in the amount of dry matter produced, as the amount of nitrogen is increased up to a certain maximum, above which additional increments of $Ca(NO_3)_2$ produce no further effect. It has been suggested by Russell (31) that the increasing effects produced by successive increments of nitrogen up to this maximum may be due to the fact that the additional nitrate not only increases the concentration of the radical in the solution, but that it also increases the extent of the absorbing surface of the roots and also that of the leaves. The process thus seems to bear some re-

semblance to autocatalysis, in which one of the products of the reaction serves as a catalyzer and hastens the reaction. This process, however, cannot go on indefinitely, because the time must come when some limiting factor will intervene and prevent further increase. Von Seelhorst (35) studied the effect upon the oat plant of the addition of nitrogen to soils containing different amounts of water. The experiment included 9 pot-cultures, which were divided into 3 series of 3 cultures each. In one series the soil used was just moist; in the second series the moisture content was slightly higher; while in the third series the soil was kept very moist. One pot in each series received no nitrogen, a second received 0.5 gm. of $NaNO_3$, and the third received double that amount. In those cultures where only a small amount of moisture was present the addition of 0.5 gm. of nitrogen was without effect, the supply in the soil being sufficient for the needs of the plants, the water supply rather than the supply of nitrogen being the limiting condition. When more water was present the plants were able to make more growth and to utilize more nitrogen. The addition of one increment of nitrogen to the slightly moister soil of the second pot increased the produce by 10 gm., but the addition of the second increment was without additional effect, the water supply having again become the limiting condition. When a liberal amount of water was supplied, the first increment of nitrogen gave an increase of 20 gm., and the addition of another increment gave a still further increase of 15.5 gm.

In the present studies, where the moisture content of the soil was kept very nearly optimum, it might be expected that the dry weight of the plants would increase, with increase in the nitrogen content of the nutrient solution, until some condition other than water-supply became the condition limiting growth. Attention has been called to the fact that this series showed a gradual increase in the dry weight of tops in the cultures of row 1, as we proceed from C1, with 1 tenth of its total osmotic concentration due to $Ca(NO_3)_2$, to C8, with 8 tenths of its total concentration derived from that salt. As we pass upward toward the apex of the triangular diagram (fig. 2), however, the effect of additional increments of $Ca(NO_3)_2$ above the amount present in Culture C1 is not so apparent and soon entirely disappears except in the case of the first increment. The results obtained in this series suggest that, in the cultures represented as lying in the upper region of the diagram, the amount of KH_2PO_4 present in the solution may be the limiting condition, beyond the second culture from the left margin in each row.

The effect of the ratio of calcium nitrate to magnesium sulphate, upon the growth of tops, is brought out by an inspection of the cultures represented as occupying row 1, at the base of the triangular diagram (fig. 1). All of the cultures in this row have the same partial osmotic concentration of KH_2SO_4; namely, 1 tenth of the total osmotic concentration.

Passing to the right from culture R1 C1, the osmotic concentration of $Ca(NO_3)_2$ increases by increments of 1 tenth, up to a partial concentration of 8 tenths of the total, culture R1 C8. As the partial osmotic concentration of $Ca(NO_3)_2$ *increases* from left to right, that of $MgSO_4$ *decreases* at the same rate. With the exception of one culture (R1 C5), the dry weight of tops increases with increasing partial osmotic concentration of $Ca(NO_3)_2$, and with decreasing partial concentration of $MgSO_4$. An inspection of harvest record (Table VI) shows that culture R1 C8, with the osmotic ratio, $Ca(NO_3)_2$ to $MgSO_4$, of 1:8 produced 100 per cent greater dry weight of tops than did culture R1 C1, where the value of the $Ca(NO_3)_2$—$MgSO_4$ ratio was 1:0.12. The greatest difference between individual cultures is found in the case of cultures R1 C1 and R1 C2, where a decrease of one increment in the partial osmotic concentration of $MgSO_4$ and a corresponding increase in the partial osmotic concentration of $Ca(NO_3)_2$ resulted in an increase of 48 per cent in the yield of tops. Increases of a similar magnitude are found when cultures R2 C2, R3 C2, and R4 C2, are each compared with the first culture in its row.

Considering the culture occupying the right margin of the diagram (fig. 3, A), all of which had the same proportion of $MgSO_4$, high yields of tops are associated with large osmotic partial concentrations of $CaNO_3$ and with low concentrations of KH_2PO_4. Proceeding toward the upper apex of the triangle, beyond the second row, the dry weights decrease with decrease in the value of the ratio, $Ca(NO_3)_2$ to $MgSO_4$. Osterhout (28) has pointed out that potassium may inhibit more or less completely the poisonous effect of excessive quantities of magnesium. Working with a marine alga (*Enteromorpha Hopkirkii*), he found these plants lived 5 times as long in a mixture of magnesium and potassium chlorides as in pure magnesium chloride, and 3 times as long as in pure potassium chloride. This same writer also grew wheat and other flowering plants in mixtures and in pure solutions of the same salts, with similar results. During a period of 40 days the wheat roots made a growth of 10 mm. in a .0937 m. $MgCl_2$ solution, but in a mixed solution (of the same total concentration) of KCl and $MgCl_2$ the growth was 153 mm. for the same time period. The results of the present investigation indicate that KH_2PO_4 is not effective in balancing the nutritive solution after a certain minimum ratio of calcium nitrate to magnesium sulphate has been reached. In this instance a marked decline in yield is shown between R2 C7 and R3 C6 as we go from a calcium nitrate to magnesium sulphate ratio of 7:1 in the former to a ratio of 6:1 in the latter.

It is obvious that if there is an optimum calcium-magnesium ratio value for the best growth of plants it can hold only within certain limits. For example, if the total concentration of the nutrient solution is too high, plants will fail to make satisfactory growth, whatever the

value of the calcium-magnesium ratio may be. Furthermore, the omission of KH_2PO_4 from the solution, or an addition of an excess of this salt, would make impossible the growth of plants in such a medium, regardless of any consideration of the calcium magnesium relation.

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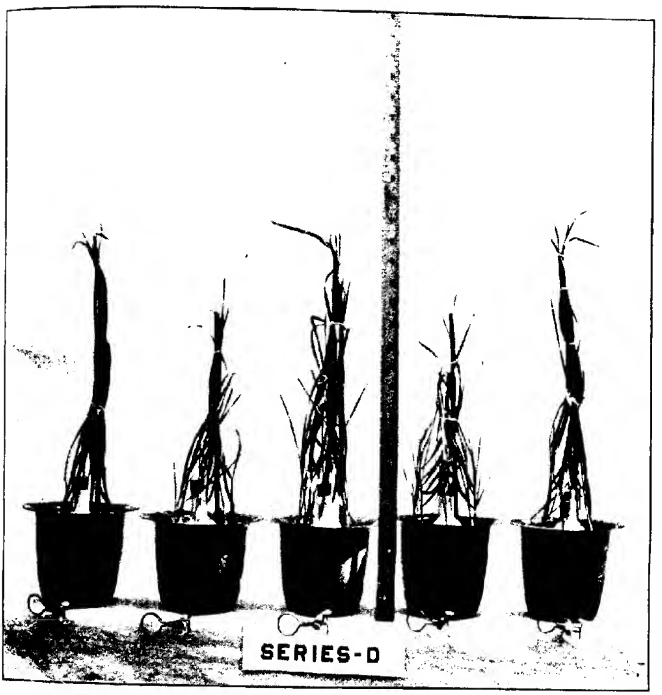
PLATE I.

Wheat cultures about 20 days old, showing form of pot and arrangement for renewing the solutions. Scale is inches.


SERIES-D

SOME BACTERIOLOGICAL STUDIES ON AGAR AGAR[1]

By

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By virtue of the ability which very small quantities of agar agar have of forming with water a comparatively non-nutrient gel, it was early used by Robert Koch in 1881, for the cultivation of certain pathogenic bacteria. Koch (22) who had long been in search of a transparent jelly, quickly recognized its value and brought it into general use a few years later. He used beef broth as the nutrient base, and with this medium cultivated a great many species of bacteria. It was he who first pointed out the great advantage of the use of solid media for the isolation and cultivation of bacteria, and with the bounteous harvest of original and important suggestions that was reaped from Koch's classical series of investigations, the new science of bacteriology attained unthought of importance, and at this period modern bacteriology may be justly said to have had its birth.

Miquel (29), in 1878-1885, had probably prior to Koch's work also used nutritive vegetable jellies in his researches on bacteria.

After the impetus given by these early workers, the use of agar as a base in solid culture media became firmly established, until to-day agar media are perhaps more used than any other. With the increase in popularity of this new substance, questions naturally arose concerning the proper methods of preparation, filtration, sterilization, adjusting the reaction and other points. Thus the period from 1882 to 1892 may be said to include problems of methodology principally. A great number of media were proposed, as well as formulae for preparation, and methods of sterilization, filtration, neutralization and the like. The subject of agar media was investigated in almost every laboratory. These investigations cannot be reviewed here as the number is too great, and besides, the greater number of the proposed methods and formulae have fallen into disuse, in the light of our modern knowledge on the subject. They are, however, of historical interest in that they show the vast amount of persevering and conscientious travail which must be encountered before any science can be said to rest on solid foundations.

Among the first to use peptone in agar media was Edington (11); Biondi (2) used the whites of eggs to clarify media in 1887. The cotton filter was probably introduced by Rosenbach (38) in 1884. Puccinelli

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(35), Mace (27), Schottelius (39), Richter (37) and many others have made notable contributions to the question of agar media. More recently Moore (30), Dominikiewicz (9) and Ravenel (36) have presented more precise and refined methods of preparing agar media. It may be said that almost every worker in the field of bacteriology employs his own peculiar mode of procedure and many times methods used successfully by one investigator fail utterly in the hands of another.

It is to be greatly deplored that such an entire lack of uniformity exists in the different steps in the preparation of the most commonly used culture media. The American Public Health Association (41) has already standardized the methods to be used in the bacteriological examination of water. Steps have also been taken to provide standard methods and media for all soil biological work, as soon as sufficient data are at hand to warrant definite conclusions.

The following data are presented in the hope that they will be of some value in the speedy attainment of these ends.

The chemical properties of agar and a proposed method of purification are discussed by the author in another paper (12). It is shown that agar agar contains considerable amounts of protein substances and other soluble nutrients. The question as to whether these nutrients are available for microörganisms is raised.

I

Can the Nutrients Contained in Agar Agar be Utilized by Microorganisms?

In the present experiment samples of ordinary commercial agar agar were washed with cold 0.5 per cent HCl solution (media usually contain more acid than this), for several hours, the filtrate evaporated to dryness and subsequently tested for the presence of Ca, Mg, S, N, Na and K. Abundant amounts of the first three elements were present, along with smaller amounts of Na and K. About 37 per cent of the nitrogen was found to be soluble. These results show that some of the nutrients present in agar agar are partly soluble in cold slightly acidulated water; probably more would become soluble in the relatively large quantity of water present in most media, if the acidity were increased in the process of sterilization at high temperatures. *Bacillus subtilis, Baccillus proteus* and several soil infusions on being inoculated into test tubes containing mother liquor of washed agar, which had been neutralized, thrived, a putrefactive odor of H_2S being given off from some of the tubes. This shows that the sulphur was used by the bacteria.

In order to ascertain whether the nitrogen present in agar agar is available for microörganisms, the agar agar itself was used as the source of nitrogen in an ammonification experiment.

Series I

One hundred grams of quartz sand, 35 gm. of powdered agar, equivalent to 134 mg. of N and 108.6 gm. of H_2O (1½ optimum) were placed in 500-c.c. Erlenmeyer flasks, and each except the check inoculated with 2 c.c. of a 1:10 soil infusion. After thoroughly mixing the contents, the flasks were incubated at about 22° C. for 7 days, and the ammonia determined by distillation with heavy Mg 0.

Series II

Two hundred grams of quartz sand, 17.5 gm. of agar (67 mg. N.), 2 gm. of glucose, 60 c.c. of water (1½ optimum) were similarly inoculated with 2 c.c. of a 1:10 soil infusion, incubated and determined as in Series I. A check flask inoculated with 2 c.c of sterilized soil infusion was also treated and estimated in the same manner.

Series III

Three hundred c.c. of water, 5.833 gm. of agar agar, and 1 gm. of dextrose, in 500-c.c. flasks were sterilized at 15 pounds pressure for 15 minutes in an autoclave. After removing and cooling to 40° C. the flasks, except the check which was inoculated before sterilization, were inoculated with 5 c.c. of 1:10 soil infusion and incubated as before. The results shown in Table I were obtained.

TABLE I

AMMONIFICATION OF AGAR AGAR

Series	Mg. N. Added	Mg. N. Converted to NH_3	Per cent Conversion
I	134.00	31.32	23.40
	134.00	24.50	18.30
II	67.00	7.77	11.60
	67.00	15.82	23.60
III	21.46	—0.14	—0.65
	21.46	0.49	2.33

The results of Series I and II show that a part of the nitrogenous matter present in agar agar can be converted by microörganisms into ammonia. The odor of putrefaction was plainly noticeable when the plugs were removed from the flasks after incubation. The conditions under which Series III was allowed to proceed approximate closely those of ordinary agar media. In this case there is little if any ammonification, but when it is considered that any decomposition which takes place must be of an anaerobic nature, except directly at the surface of the flask, such results are to be expected. They show that little if any nitrogen is made use of by microörganisms under the conditions of the experiment.

It is of passing interest at this point to merely mention the work of the Pringsheims (34). They showed that agar agar may be a source of energy in the assimilation of atmospheric nitrogen. In a 0.4 per cent dextrose and 0.2 per cent agar agar mixture, inoculated with *Bacillus gelaticus* and *Bacillus clostridium*, 26.6 mg. N. were assimilated per gram of agar agar; where .005 per cent $(NH_4)_3PO_4$ was substituted for the dextrose, the amount fixed was 15.4 mg. Similar results were obtained with *B. gelaticus* in association with *Azotobacter chroococcum*. These results show the origin of the nitrogen required by vegetable and animal organisms in the sea as both Azotobacter and Clostidria occur in sea water, and that the agar present in the red and brown algae is rendered available as a source of energy with the aid of *B. gelaticus*.

B. gelaticus is a deep sea organism capable of liquefying agar by means of the enzyme gelase. It is fully described by Gran (15).

To test out further the question of the availability of the salts present in agar, slants of non-nutrient agar, made up with distilled water, were inoculated with the following organisms: *Bacillus Rutida, Bacillus vulgatus, Bacillus megatherium, Bacillus subtilis, Bacillus butrycus, Bacillus mesentericus, Bacillus prodigiosus, Saccharomyces* (sp ?). *Penicillium viridicatum* Westling and *Trichoderma Koningi*. Examinations of the tubes after 26 days showed that all the organisms except, *B. butrycus, B. mesentericus* and possibly *B. vulgatus*, had grown to a greater or lesser extent. The yeast, the two fungi and *B. subtilis* grew noticeably better than the others, which fact would seem to indicate that the higher organisms are better able to extract food from agar than bacteria. The culture of *B. prodigiosus* which was used, formed a deep red color on ordinary media, but on non-nutrient agar no pigment at all was produced. This organism is especially susceptible to changes in environment. Luckhardt (26) shows its variability in pigment production on different media. When grown in an atmosphere of CO_2 it is colorless. Duggar (10) states cultures may lose their pigment when grown in acid media. It has been the author's observation that the pigment is much deeper on alkaline than on acid media.

The culture of *B. subtilis* was found on microscopic examination to consist entirely of spores. This observation is akin to that of Stephandis (42), who found that spore formation in *Bact. anthracis* increased as the media became poorer in nutrients.

This experiment indicates that although microörganisms do not thrive well on non-nutrient agar, yet they are able to grow and multiply on it to some extent.

Summarizing, the whole experiment shows that although there are considerable amounts of soluble nutrients including nitrogenous substances in agar, they are used by microörganisms under ordinary conditions, only

to a very limited extent. If the conditions are made favorable however, microbial activity becomes fairly marked, one of the results being a transformation of a part of the protein into ammonia. It is advisable at any rate to use a high grade or purified agar in refined bacteriological work.

METHODS

Before passing on to the following experiments a brief resumé of the methods employed will be inserted here.

In general two media were used as follows:

I. Peptone—KCl Agar (Chamot)	
Agar	20 gm.
Peptone	20 gm.
KCl	6 gm.
Tap water	1000 c.c.
Acidity at 50° C.	1% HCl

II. Lipman & Brown's (25) Modified Synthetic Agar	
Agar	15 gm.
Peptone	.05 gm.
K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
Dextrose	10 gm.
$Fe_2(SO_4)_3$	Trace
Distilled Water	1000 c.c.
Acidity unadjusted.	

Care was taken to prepare the media always in the same way so as to get as little variation as possible between different batches. Leitz's "shred agar" and "peptone" were used throughout the investigation. The agar was washed for several hours in about one-half the amount of water to be finally added to the medium. The water was usually changed several times. The finely cut, swollen shreds were separated from the water by filtration through cheesecloth, made up to weight with water, and the other ingredients added. Titrations were made at 50° C., as this temperature is nearer that of incubation, and because peptone media become more acid on heating. At first egg albumen was used to clarify the medium but this was afterwards deemed unnecessary, besides consuming considerable time. The tubed media were usually liquefied by means of flowing steam in an autoclave, after which the tubes were placed in a large water bath kept at 45° C. This procedure reduces to a minimum any variations in the temperature of the medium at the time of pouring the plates. Dilution water containing the bacteria was never allowed to remain exposed in the plates, for more than a few minutes. The period allowed for development of colonies of bacteria was 120 hours. Plates were poured in series of five, as in the author's opinion that number is desirable to obtain truly comparable data. In reporting the results, four plates are given, as it was found that that number usually check reasonably closely. For soils the usual dilutions used were 1:50,000 and 1:100,000. In the examination of soil, 20 gm. was placed in 200 c.c. of sterile tap water and shaken vigorously for five minutes. Dilutions made from this infusion were shaken for one minute each.

The soil and water samples were obtained on or near the College Farm, and were chosen so as to include as large a variety as possible.

Soils

I. Penn sandy loam (acid).
II. Sassafras silt loam (slightly acid).
III. Penn loam (red shale) (neutral).
IV. Alloway clay (very acid).
V. Penn sandy loam (neutral).
VI. Sassafras gravelly loam (neutral).

Water Samples

I. Ground water (Campus spring).
II. Surface water (Campus pond).
III. Surface water (Reservoir).
IV. Ground water (shallow well).

II

Comparison of Counts on Ordinary and Purified Agar

The purified agar described in another paper (12), was compared with ordinary agar in media made up in the same way. The essential steps in the process of purification are first washing the crude agar agar with dilute acetic acid, and finally with water, then dissolving in hot water to make a 5 per cent solution. This is precipitated by an excess of alcohol or acetone, filtered, washed and dried.

TABLE II
COMPARISON OF "PURIFIED" WITH "ORDINARY" AGAR[1]

Title	Soil I		Soil IV	
	Purified Agar	Ordinary Agar	Purified Agar	Ordinary Agar
MEDIUM I				
Plate I	3.95	3.80	2.15	2.20
Plate II	4.10	4.00	2.50	2.25
Plate III	4.30	4.05	2.10	2.40
Plate IV	3.60	4.25	2.35	1.90
Average	3.99	4.02	2.27	2.19
MEDIUM II				
Plate I	6.40	6.75	2.65	3.00
Plate II	6.40	5.95	2.50	3.10
Plate III	5.80	6.20	3.15	2.60
Plate IV	6.15	6.50	3.00	3.30
Average	6.35	6.19	2.82	3.00

[1] Bacteria are expressed in millions per gram of soil.

The same weight of both purified and ordinary agar agar was used. This introduces a slight error due to the small increase in concentration in the case of the purified product. Two soils and two media were used in this experiment, the data of which are given in Table II.

The data show that there is little if any difference between the media made up with ordinary and with purified agar agar. Perhaps there is a slight tendency in favor of the unpurified agar medium but this may be due to either the soluble nutrients in the agar or else to an antiseptic action caused by the presence of a small quantity of alcohol which may not have been removed in the process of washing. The purified agar media were much the clearer, thereby making the colonies more readily distinguishable and consequently easier to count.

Agar agar, purified in the manner suggested, has no appreciable effect on the bacterial counts from soils as compared with ordinary agar agar.

III

The Influence of Varying Concentrations of Agar agar in Culture Media on Bacterial Counts

Among the many unsolved problems appertaining to bacteriological methods and the preparation of culture media is that of the proper concentration of agar which will give the best results. At the present time all sorts of concentrations extending from 1 to 2.5 per cent are usually employed. It is the purpose of this work to throw some light on the influence of agar-concentration in media, on bacterial counts. Dr. E. M. Chamot of Cornell University first brought this question to the writer's attention, with regard to the sanitary examination of water.

Very little research has been done on the question of agar concentration in media. Hesse (17) showed that the size of the bacterial colonies developing on gelatin was not larger than those on 1 per cent agar. Gelatin plates reached their maximum development in 6 to 10 days, while it required 11 to 15 days for agar plates. A greater number of organisms grew on agar than on gelatin.

Hinterburg and Reitman (21) determined the effect of varying concentrations of agar on the growth of *Pseudomonas pyocyaneus*. A very slight change in concentration caused much change in the vitality and character of the growth of this organism.

Gazetti (14) has pointed out the marked effect of composition of media on the growth of chromogenic bacteria.

Heim (16), Moore (31), Fisher (13), Conn (6) and others have recommended about 12.5 gm. of agar per liter of medium. Lipman and Brown (25), Temple (44), Cook (7) and a host of other investigators have used a concentration of 1.5 per cent agar. The committee on Standard Methods for the Examination of Water and Sewage (41) report that 10 gm. of agar per liter of medium, appears to give higher and more consistent counts than the use of greater amounts.

Wolf (48) experimented on the growth of a number of bacteria on several media with varying concentrations. He states that most bacteria

grow well at a concentration of 50 per cent dry matter in the nutrient medium, but the growth is poor in media containing 60 per cent dry matter. He makes the observation that the pigment forming bacteria appear to thrive best on the more concentrated media.

TABLE III

EFFECT OF CONCENTRATION OF AGAR ON BACTERIAL COUNTS[1]

	MEDIUM I						
in Media Per cent	0.5	1	1.5	2	2.5	3.0	4.0
Soil I	3.80	4.35	5.00	5.45	5.60	4.70	5.30
	3.20	4.20	5.30	5.10	4.80	5.60	4.60
	3.10	3.80	4.40	4.75	4.50	4.80	4.80
	3.63	4.70	4.55	5.20	4.70	5.05	5.25
Average	3.431	4.26	4.81	5.12	4.90	5.04	4.99
Soil II	3.90	7.80	6.75	7.75	6.85	6.70	6.60
	4.05	7.40	7.25	7.80	6.60	7.35	6.50
	4.50	7.50	7.35	7.10	7.05	6.75	6.90
	3.60	7.70	7.50	6.75	6.40	7.00	6.00
Average	4.01	7.60	7.21	7.35	6.73	6.94	6.50
Soil III	7.50	7.45	8.55	8.40	5.75	4.35	6.00
	6.50	8.30	8.50	8.50	5.10	6.10	6.50
	5.50	8.25	8.05	10.00	6.35	6.25	5.76
	5.75	8.55	8.35	8.00	6.50	6.40	5.50
Average	6.31	8.14	8.36	8.73	5.93	5.78	5.94
Soil IV	12.30	13.80	13.20	12.90	12.60	12.30	10.20
	10.80	13.20	13.80	12.60		12.30	10.50
	11.70	11.70	13.20	15.00	12.00	12.00	9.00
	9.90	12.60	15.60	16.50	12.30	10.20	9.90
					12.90		
Average	11.18	12.83	13.95	14.25	13.20	11.70	9.90

[1] Bacteria are expressed in millions per gram of soil.
Dilutions 1:50,000.

Besides the researches just reviewed, there are innumerable recommendations from every side, as to the concentration of agar in media. All amounts from 4.5 gm. per liter to 40 gm. per liter appear to have been used, but no systematic researches have been discovered in the literature touching upon a comparison of different concentrations, with different media and a number of soils. Since agar agar always has a certain amount of moisture (about 16.5 per cent), it can be seen that unless it is dried an error will be introduced in weighing out the exact amount desired. But the moisture in agar agar samples from different sources is constant or nearly so (within 3 or 4 per cent at most); hence the error is not great. Plain washed agar was used in this experiment.

The data in Tables III and IV, graphically represented in figure 1, give the results obtained in the course of this investigation.

Discussion of Results

An inspection of the data, curves and plates shows a gradual increase in numbers of bacteria from an agar concentration of 0.5 per cent to about 1.5 to 2.0 per cent where the maximum is reached. In concentrations above 2 per cent agar the general trend of results is a decrease in numbers. The 0.5 per cent agar solidified perfectly, but it allowed the colonies to develop faster, hence at the end of 120 hours they had attained a very large size. This fact would tend to keep down the numbers of bacteria developing on this medium. The lower concentrations of agar appear to be more favorable to certain species than to others, an inspection of Plates I-III will bear out this statement. Such a medium may be uti-

TABLE IV

EFFECT OF AGAR CONCENTRATION ON BACTERIAL COUNTS[1]

Per cent Agar in Media	0.5	0.75	1.0	1.25	1.5	2.0	3.0	4.0
Soil V.....	4.10	4.65	6.40	6.25	7.35	6.50	6.70	6.50
	4.60	4.50	6.00	6.50	7.50	6.75	6.60	6.50
	4.65	5.00	5.90	6.85	7.65	7.25	6.50	7.50
	4.50	4.00	5.40	7.75	6.50	6.40	7.00	8.00
Average	4.46	4.54	5.93	6.81	7.25	6.75	6.70	7.13
Soil VI.....	7.00	7.00	9.50	13.50	15.00	12.50	14.50	14.50
	6.80	7.00	9.20	13.00	15.10	14.20	13.50	13.00
	7.00	7.20	10.00	13.50	13.00	13.70	13.50	14.00
	6.40	lost	8.10	14.40	16.00	13.00	lost	13.30
Average	6.80	7.07	9.20	14.10	14.78	13.35	13.83	13.70

[1] Bacteria calculated in millions per gram of soil.
Dilutions 1:50,000.

lized in the study of the cultural characteristics of certain bacteria which form colonies too minute to be easily studied on more concentrated media. One per cent concentration of agar allowed more and larger colonies to develop, but it is open to the same objections as the 0.5 per cent agar. It does not allow a maximum colony development of the bacteria which are present. The species, too, seem to be restricted, as in the lower concentrations. There is but little difference between the 1.5 per cent and 2.0 per cent agar so far as counts alone are concerned, but since the colonies are larger, the species apparently the same, the greater ease in pouring and counting the plates, and finally the use of less agar, lead the author to the conclusion that 1.5 per cent agar is the best under the conditions of the experiment. Concentrations higher than 20 gm. of agar per liter of medium (2 per cent) form such a hard mass in the plates that the colonies are very small and some fail to develop at all. Such a medium may

be of value in cases where long storage is necessary, as it does not lose water by evaporation very readily. Other objections to high agar concentrations are the difficulty in filtration, pouring the plates, counting and identifying the species, and finally the added expense of the agar itself.

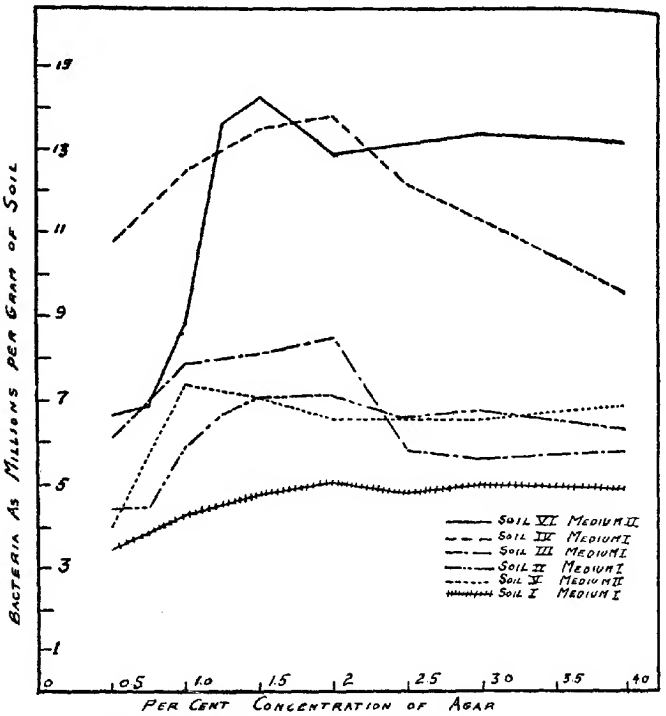

Fig. 1.—Diagram showing the effect of agar concentration on bacterial counts.

The same results hold with both the media tried, and with the several soils. Additional concentrations of 0.75 per cent and 1.25 per cent were tried in the examination of Soils V and VI. The 1.25 per cent concentration gave very good results, the colonies being of good size, the species well represented and the medium firm and hard. There is little difference between 1.25 per cent and 1.5 per cent agar. The 0.75 per cent agar has the same undesirable features as the 0.5 per cent, yet it may be useful in the determination of cultural characters of certain bacteria, and especially the actinomyces, streptothrices and some fungi. Agar of low concentrations may have a practical application in the culture of *Pseudomonas radicicola* for inoculation purposes, as a much larger growth will develop than on more concentrated substratum.

To test out this point further, pure cultures of *Bacillus coli, Bacterium mycoides* and *B. subtilis* were inoculated on agar slants in test tubes and on streaks in Petri dishes. In the case of all three bacteria the growth was more abundant on the media of low concentration of agar, but this heavy growth took place at the expense of the latter's characteristic appearance. *B. coli* incubated at 30° C. grew best at 1.5 to 2.0 per cent agar concentration, the growth becoming more uncharacteristic as the stiffness of the medium increased. *Bact. mycoides,* on the other hand, was much more characteristic in appearance at lower concentrations of 0.75 and 1.25 per cent agar. *B. subtilis* grew equally well on all the media, but the wrinkly appearance did not show up well except at concentrations of 1.5 per cent or over.

Summary. On the whole a medium having an amount of agar equivalent to 12.5 to 15 gm. per liter has been found to be the best in this series for the following reasons.

1. It permits of a maximum development of colonies. 2. The species are better represented than on lower concentrations and they form more characteristic colonies than at higher concentrations. 3. The medium is more easily filtered, melted and poured into plates than agar of greater concentrations. 4. The well formed colonies permit of very accurate counting. 5. Since agar has been shown to contain many impurities, these are correspondingly reduced with the concentration. 6. Less agar is required.

Low concentrations of agar may be of value in the study of cultural characteristics of certain microörganisms, due to large colony development.

High agar concentrations may be utilized, either where long storage is necessary, or for very slow growing organisms.

The Influence of the Acidity of the Medium on the Number of Bacterial Colonies Developing on Plates

The question of the proper reaction of media for bacteriological examination of soil and water is of great importance. Some standard reaction should be used in the ordinary examination of soil, milk and water for microörganisms. Of course the optimum reaction is not the same for the different kinds of microörganisms, as bacteria, yeasts and fungi, and even different species of bacteria or fungi thrive best in media of a particular acidity or alkalinity. For example *Bacterium mallei* grows best at an acidity of about 2.5 per cent. Most bacteria could not endure so much acid and would not develop on such a medium. On the other hand *Bacterium anthracis* and most of the streptothrices prefer an alkaline medium. The problem is then one which is impossible except in so far as a medium of such reaction must be used which will allow the development of the greatest number of species from the substance under examination.

An acidity of + 1 (i. e. 10 c.c. Normal HCl per liter of media) is most often used by bacteriologists. This is the reaction recommended by the American Public Health Association Committee on Methods for the Examination of Water and Sewage (41). They allow an error of ± 0.2 in the final reaction. Considerable work has been done on the question of reaction of media. Most of it is either antiquated, as for instance the use of litmus or other unreliable indicators in the titration, or else of minor importance. Only a small part of the literature will be reviewed here.

Some of the investigators recommending alkaline media are Hiltner and Stömer (20), Thomann (46), Deeleman 8), and Hesse and Niedner (18). In their opinion an alkaline medium is preferable in soil or water examination. Others preferring neutral media are Heim (16) and Schultz (40). Lipman (24) and Christensen (4) believe slightly alkaline media are best for Azotobacter, while Wright (49) avers anaerobes as a rule prefer a medium which is near the neutral point. Temple (44) states that the optimum reaction for media in soil work is between the neutral point and 0.5 per cent acid. Cook (7) uses a reaction of + 0.25 in his urea—NH_4NO_3 agar with excellent results. Conn (6), who has made a very thorough study of media for soil examination, proposed a sodium asparaginate agar, with a reaction of 0.8 per cent HCl. He showed that a medium with an acidity of 0.8 per cent HCl gave about double the number of colonies as one with an acidity of 1.5 per cent HCl. He did not try any acidities below 0.8 per cent for fear of volatilizing some of the ammonia from the NH_2 H_2PO_4 which he used in this medium. For his soil extract gelatin, however, a reaction of 0.5 per cent acid was used.

Lipman and Brown (25) showed that the neutralization of a part of the acidity of their synthetic agar, greatly reduced the number of colonies developing on the medium. This medium owes its acidity mainly to the K_2HPO_4 which is used. The author has determined the reaction several times with the result that an average acidity of about 0.4 to 0.5 per cent was found. A simple change of reaction does not satisfactorily account for the large decrease found, hence some other factors must enter into the problem. Petri and Maassen (32) have pointed out that tertiary phosphates are harmful to most bacteria, and assuming the following equation, tertiary phosphates are formed when the acidity is neutralized;

$$NaOH + K_2HPO_4 = NaK_2PO_4 + H_2O$$

or since $MgSO_4$ is present possibly some Mg would replace the alkalies with the formation of a magnesium phosphate, which may be precipitated and hence would be of no value to the bacteria. Their results at least may be explained on the theory either that the tertiary phosphates are deleterious to bacteria or else that the precipitation of some of the nutrients occurs.

Series I

In order to determine approximately the optimum reaction of agar media for the purpose of obtaining the largest bacterial counts on plates, a series of soils and waters were quantitatively examined by the plate method. On account of the complexity of salts present in synthetic media, an ordinary peptone—KCl agar (Medium 1) was used in this work.

Each batch of media was carefully prepared at the same time, thoroughly mixed, placed in flasks and the reaction adjusted by the titration of 10 c.c. of the medium at a temperature of 50° C. with N/20 NaOH, phenolphthalein being used as the indicator. The temperature at which the titrations were made was 50° C. because peptone solutions are decidedly more acid at higher than at lower temperatures. Some error is introduced by titrating at 50° C. but the change in acidity from 50° C. to incubation temperature is slight compared with that between 100° C. and 50° C.

Media differing by 1 per cent were made up from — 5 per cent NaOH to + 5 per cent HCl. It was found that agar containing more than 2 per cent HCl fails to solidify if it is sterilized in an autoclave under pressure. To overcome this difficulty the following procedure was used. The medium, as soon as it was made up and while still boiling hot, was adjusted in reaction. The average of duplicate 10-c.c portions removed, cooled to 50° C. and titrated, was used to determine the reaction. After adjusting the reaction of all the flasks and thoroughly shaking, the flasks were cooled to 45° C. Then 9-c.c. portions were pipetted out into the sterile Petri dishes containing the inoculum. The usual precautions against contamination were observed during the whole process. By this procedure the agar solidified very well up to 4 or 5 per cent acid. This method however was utilized only in the case of media which would not otherwise harden. Five plates were poured, the four best checks being reported.

Discussion of Results. Upon examining the curves in figure 2 compiled from Table V it is seen that their general appearance is the same, i. e. three out of the four reach a maximum at the neutral point, the fourth (Soil IV) reaching its greatest height at + 1. This shows that the best colony development takes place at a neutral or slightly acid reaction. Very few bacterial colonies developed at acidities greater than + 2, yet the small but fairly constant number that were invariably present, appear to constitute a peculiar group of acid-resistant bacteria. The + 5 medium was rather crumbly and soft, making the colonies difficult to see and for this reason too much emphasis cannot be placed on the number of colonies found on this medium. An acidity of 1 per cent inhibited a maximum colony development in three out of four cases. It is interesting to note that Soil IV, which gave the highest counts at + 1 is very

acid; this may be only a coincidence, but it is the only soil where the + 1 medium is superior to the neutral. Fungi and actinomyces were particularly abundant on all the acid media, several species of Trichodermae, Cladosporia, Penicillia and Aspergilli persisting even on the + 4 and + 5 media. Alkaline media although inferior to neutral or slightly acid do not appear to be as toxic, as a whole, as the more acid media. The size of the colonies appears to increase as the neutral point is approached from either direction. Few colonies develop on — 4 and — 5 media. The

TABLE V

INFLUENCE OF REACTION OF MEDIA ON BACTERIAL COUNTS

SERIES I

Bacteria per gram of soil or per cubic centimeter of water

Reaction	+5.0	+4.0	+3.0	+2.0	+1.0	Neut'l	—1.0	—2.0	—3.0	—4.0	—5.0
Soil II...	0	100	400	600	2,500	5,000	3,500	2,250	1,700	1,250	500
Dil. 1:50,000	0	200	350	600	3,000	4,250	3,700	2,050	1,450	1,400	700
	50	200	200	400	2,800	3,600	4,050	2,500	1,750	1,000	700
	100	250	350	600	2,350	4,500	3,750	1,700	1,700	1,100	800
Average ...	75	187	325	550	2,660	4,340	3,750	2,130	1,650	1,190	675
Soil I...	50	100	100	400	2,050	2,600	1,350	950	450	100	100
Dil. 1:50,000	0	100	100	300	2,200	2,500	1,550	1,000	500	150	150
	0	50	200	450	2,050	2,550	1,850	1,350	400	300	50
	0	0	100	500	2,500	2,320	1,800	1,050	600	100	150
Average ...	13	63	125	413	2,200	2,492	1,640	1,090	587	163	112
Soil IV...	0	0	150	350	3,500	2,500	2,300	1,300	650	300	100
Dil. 1:50,000	0	0	100	200	3,450	2,800	1,900	1,300	750	250	150
	0	0	100	250	3,000	2,750	2,000	1,000	500	400	50
	0	50	50	150	3,200	3,300	1,600	1,100	900	400	50
Average ...	0	13	100	238	3,300	2,840	1,950	1,180	700	338	88
Water I											
Bac. per c.c.	0	50	60	160	240	600	160	80	80	60	20
Dil. 1:20 ..	0	20	40	180	400	540	200	120	40	60	40
	0	30	60	200	300	460	240	100	40	40	40
	20	40	lost	200	340	440	280	60	80	20	0
Average ...	5	35	55	190	350	410	220	90	60	45	25

Note:—Thousands omitted in case of Soils I, II, and IV.

addition of much alkali to agar causes it to darken, thus making the counting of the plates difficult. Streptothrices grow better on neutral and alkaline media while on the other hand alkalies inhibit the development of fungi. In the three cases where the neutral media gave the highest counts the increase over the 1 per cent acid agar is about 20 per cent. The size of the colonies on the very acid or alkaline media is as a rule very small, the size, pigmentation and the individual colony characteristics become more conspicuous near the neutral point.

Series II

To check up this work and confine the limits of acidity and alkalinity more closely, another series of plates was poured, in which the reactions used were from + 1.5 to — 0.5.

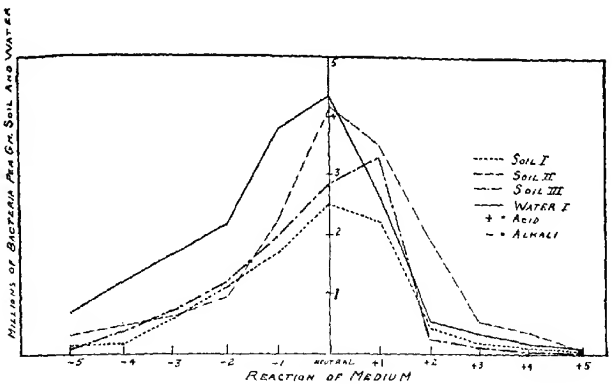

Fig. 2.—Diagram showing the influence of reaction of media on bacterial counts (Series I).

The results follow in Table VI, the averages being graphically represented in figure 3.

Discussion of Results. The data contained in Table VI and the graphs drawn from this in figure 3, confirm in general the first experiment. Beginning with an alkalinity of — 0.5 there is a large increase in the number of colonies per plate, up to the neutral point, then there is a comparatively constant decrease, except in one case, from neutral to + 0.5, but all acidities tested greater than this, are decidedly injurious to the development of bacterial colonies. There is not a very marked difference in the numbers of colonies on neutral and + 0.5 media. Averaging the results of the three soils the neutral medium shows an increase in bacterial colonies over the + 1 per cent acid medium of 18 per cent. In the case of the two waters there is a 12 per cent increase for the neutral medium over the + 0.5 and about 17 per cent over the + 1.0 media.

Water Sample III shows a depression at + 0.5, but this is plainly an exception to the general trend of the results.

From this experiment the conclusion may be drawn that with the media used, most bacteria from soil or water develop best at a neutral or slightly acid reaction. Probably + 1 is too acid for the development of maximum numbers of bacteria. This together with the more characteristic growth of colonies and greater freedom from fungi, are advantages to

be gained by the use of a neutral or slightly acid medium. It may be that lack of systematic work along this line has led to the usual recommendation of media which is too acid to give the best results. Also, as has been pointed out before, peptone media have a greater apparent acidity in hot than in cold solutions, hence the cooled solution is considerably less acid than is supposed from the titration at the higher temperatures.

TABLE VI

INFLUENCE OF REACTION OF MEDIUM ON BACTERIAL COUNTS[1]

SERIES II

Reaction	+1.5	+1	+0.5	Neutral	−0.5
Soil III.....	1.30	4.10	5.00	5.40	1.50
	1.65	4.10	4.90	5.50	1.80
	1.25	4.90	4.80	6.25	1.45
	lost	4.50	5.50	5.25	2.00
Average	1.40	4.40	5.05	5.58	1.70
Soil V.....	2.95	4.50	5.25	5.50	3.60
	3.00	4.30	5.05	5.60	3.20
	3.25	3.95	5.50	6.00	3.00
	2.60	4.70	4.70	6.35	3.20
Average	2.95	4.36	5.13	5.86	3.25
Soil I.....	1.50	2.10	2.50	2.10	1.30
	1.30	2.00	2.60	2.00	1.25
	1.15	1.60	2.30	1.80	1.50
	1.25	1.90	2.80	2.50	1.50
Average	1.30	1.90	2.55	2.10	1.38
Water II.....	600	850	980	1060	750
Dilution 1:10	500	700	850	1020	800
	480	720	900	950	760
	520	lost	800	920	680
Average	530	760	880	990	750
Water III.....	340	760	850	1040	450
Dilution 1:10	360	920	780	980	400
	330	900	760	900	470
	330	870	780	940	470
Average	340	860	790	970	450

[1] Millions omitted in case of Soils I, III and V.
Soil dilutions 1:50,000.

The medium which was titrated boiling hot and adjusted to a reaction of + 1, on cooling to 23° C. was found to be 0.75 per cent acid. In special cases as in water examination, where bacteria of the "coli" group are especially sought for, an acidity of + 1 may be justified. Since practi-

cally all waters are alkaline and most soils only slightly acid, the above findings seem to be in harmony with the natural conditions (See Plate IV, fig. 1).

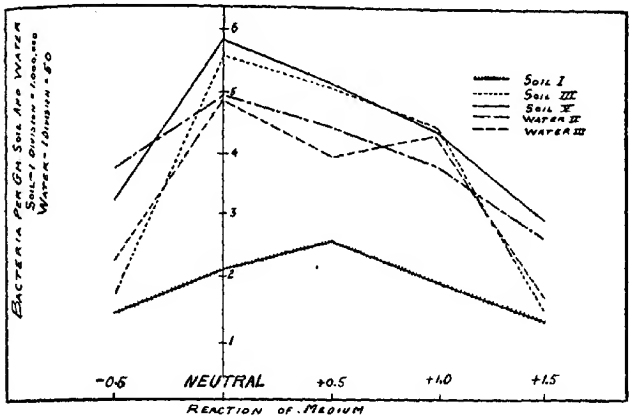

Fig. 3.—Diagram showing the influence of reaction of media on bacterial counts

Series III

A number of bacteria and fungi were inoculated on agar slants and as plate streak cultures, made up with media of varying reaction. Table VII gives the relative growth of the several bacteria tested. The data on the fungi are given later.

TABLE VII

INFLUENCE OF REACTION ON GROWTH OF BACTERIA

Reaction	Bact. mycoides	B. subtilis	B. fluorescens liquefaciens	B. coli	B. prodigiosus
−4	No growth	Moderate	No growth	Very feeble	Feeble
−3	Very feeble	Moderate	No growth	Very feeble	Moderate
−2	Feeble	Vigorous	Feeble	Feeble	Moderate
−1	Moderate	Vigorous	Moderate	Moderate	Vigorous
N	Vigorous	Vigorous	Vigorous	Moderate	Vigorous
+1	Moderate	Vigorous	Moderate	Vigorous	Moderate
+2	Feeble	Vigorous	Feeble	Moderate	Feeble
+3	Very feeble	Feeble	No growth	Feeble	Very feeble
+4	No growth	Feeble	No growth	Very feeble	No growth

Although the table gives only a limited amount of data, yet it is interesting in that it shows the most favorable reaction for the growth of the organisms tested. In most of them there is a considerable range where the growth is good, for example *B. subtilis* grew on all the media tested. *Bact. mycoides* and *B. prodigiosus* seemed to prefer neutral or

slightly alkaline media, *B. fluorescens liquefaciens* grew best on neutral, and *B. coli* best on media of an acid reaction. Another point brought out by this experiment is the change in character of the growths, pigment production, etc., which occur when the bacteria are grown on media of different reactions. For instance *B. prodigiosus* produced a very dark red pigment on alkaline and neutral media, and at acidities over 2 per cent there was little or no color.

The well known thick wrinkly growth of *B. subtilis* appeared only at reactions between -3 and $+2$. On the more alkaline media it was smooth and slimy, while on very acid media the growth was scanty and had a dried appearance. *B. coli* is adapted to a wide range of acidity and alkalinity, the growth probably being most vigorous at $+1$. *Bact. mycoides* grew poorly at acidities greater than $+1$, the growth being of a dirty gray color, amorphous in structure and entirely devoid of the characteristic rhizoidal appearance. Microscopic examination of the cultures revealed no striking morphological changes, except that in the case of *B. subtilis* after 10 days there were vegetative forms of the organism only on the -1 and neutral media.

These results show the importance of making culture media always of the same reaction, due to the variability of many bacteria to slight reaction changes. Lipman (24) has suggested a practical application of this phenomenon, in the approximate determination of soil acidity.

IV

Influence of Reaction of the Medium on the Number of Fungi Developing on Plates

It is generally conceded that fungi desire a more acid medium for best development, than do bacteria. But little experimentation has been conducted on the effect of reaction on fungi. Thom (45) has shown that most species of Pencillium do not thrive well on alkaline media, but that the best reaction is from neutral to an acidity of N/10 HCl. Different acids have very different actions on fungi. Traaen (47) states that an acidity of from N/150 to N/50 is most suitable for the majority of fungi. Trichoderma is especially resistant. H_2SO_4 is not as toxic as HCl which in turn is not as toxic as HNO_3. Marchal (28) states that soils having a reaction from neutral to alkaline are not favorable for fungi. Kopeloff (23) found that certain species of Rhizopus, Zygorhynchus, and Penicillium have as a rule a narrow range of reaction tolerance as regards ammonification activities. This lies between the neutral point and an acidity equivalent to 2000 pounds CaO per acre. Greater acidities than this caused a depression in ammonifying power, as did an increase in alkalinity. Coleman (5) working with a number of soil fungi found that they were adapted to a wide range of reaction tolerance, but that their activities were suppressed in alkaline or very acid media.

The medium used in the present work to determine the best reaction for growth of soil fungi is one proposed by Mr. G. P. Koch, one of the author's colleagues, in unpublished data. It consists of,

Agar—20 gm.	K_2HPO_4—.25 gm.
Glucose—20 gm.	$MgSO_4$—.25 gm.
Peptone—5 gm.	..Distilled H_2O—1000 c.c.
NH_4NO_3—1 gm.	Reaction + 1

The reaction was varied from —1 to +2 by steps of 0.5 per cent. The +1.5 and +2 media did not harden after sterilization, hence the method already described to cause the agar to harden was resorted to. This medium gave good results, the colonies of fungi being well separated, and distinct. The growth of mucors and streptothrices was also inhibited. The counts given in Table VIII, and represented in figure 4 were made after five days' incubation.

TABLE VIII

INFLUENCE OF REACTION OF MEDIUM ON COUNTS OF SOIL FUNGI[1]

	+2.0	+1.5	+1.0	+0.5	Neutral	—1.0
Soil IV..... Dil. 1:10,000	120	150	160	70	90	50
	140	120	120	80	60	40
	110	160	150	90	50	30
	120	140	120	80	60	lost
Average	123	143	138	80	65	40
Soil II..... Dil. 1:10,000	40	80	100	90	40	40
	60	60	90	60	60	40
	90	90	70	60	40	60
	Mucors	Mucors	80	60	60	Mucors
Average	63	77	85	68	50	47
Soil V..... Dil. 1:10,000	70	130	120	100	90	70
	90	130	120	90	60	50
	110	140	150	100	90	30
	80	100	110	lost	90	50
Average	88	125	125	97	83	50

[1] Fungi represented as thousands per gram of soil.

Discussion of Results. Although somewhat incomplete, these results show that the optimum reaction for the development of soil fungi is more acid than the bacterial optimum. There is little difference between the counts on the media whose reactions were respectively +1 and +1.5. Alkaline or neutral media do not seem to be as favorable as those with an acid reaction. An acidity of +2 is too high and +0.5 is too low for a maximum development of colonies as a glance at the curves will indicate.

It is generally believed more fungi are present in acid soils than in those of a neutral or alkaline reaction. Soil IV is extremely acid while

Soils II and V are nearly neutral. The data seem to support this belief. As in the experiment with bacteria, a number of genera of soil fungi were tested with media of varying reactions. Table IX gives a summary of the results.

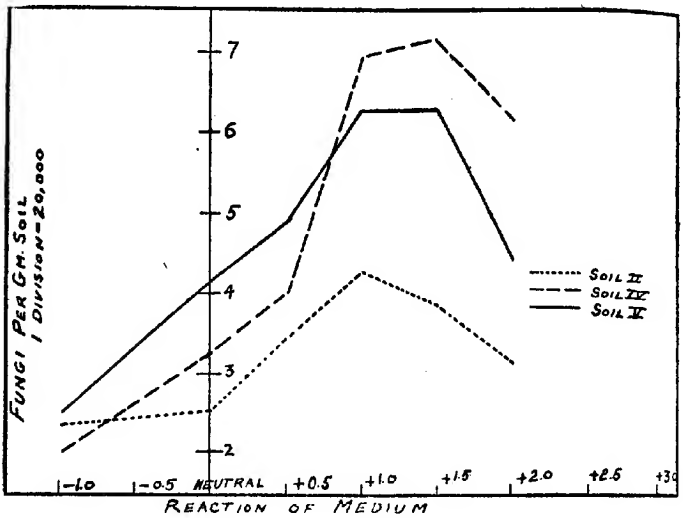

Fig. 4.—Diagram showing the influence of reaction of media on counts of soil fungi.

As a whole the acid media were the more favorable for all the fungi tested. *Aspergillus niger* grew and sporulated at all the reactions at

TABLE IX

INFLUENCE OF REACTION ON GROWTH OF SOIL FUNGI

Reaction	Aspergillus niger	Cladosporium epiphyllum	Penicillium viridicatum W.	Trichoderma (sp. III)
—5	Very feeble	Very feeble (pigment)	No growth	.....
—4	Feeble	Feeble (pigment)	No growth	Feeble
—3	Feeble	Moderate (deep pigment)	No growth	Moderate
—2	Moderate	Vigorous (pigment)	Moderate	Vigorous
—1	Moderate	Vigorous (pigment)	Vigorous	Vigorous
N	Vigorous	Vigorous (pigment)	Vigorous	Vigorous
+1	Vigorous	Vigorous (pigment)	Vigorous	Vigorous
+2	Vigorous	Moderate (pigment)	Moderate	Vigorous
+3	Moderate	Moderate (slight pigment)	Moderate	Feeble
+4	Moderate	Moderate (very slight pigment)	Feeble	Very feeble
+5	Moderate	.....	.....	.....

which it was grown. *Penicillium viridicatum* W. was also very resistant to acid but sensitive to alkali. *Cladosporium epiphyllum* produced a dark coloration of the media, which became fainter as the acidity increased.

Many instances are on record where fungi have been observed growing in fairly concentrated acid solutions. An instance of a Penicillium apparently thriving in a bottle of N/10 HCl was noticed by the author. Such phenomena may be explained on the theory that the fungus uses the acid only as a medium in which to develop, and obtains its food from the foreign substances which are present.

Summary of Acidity Experiments

Under the conditions of the experiment:

1. The optimum reaction for the development of maximum numbers of soil and water bacteria is from the neutral point to an acidity of + 0.5.
2. For soil fungi the optimum acidity is from + 1 to + 1.5.
3. Many soil bacteria and fungi are able to develop at comparatively high concentrations of acid and alkali.
4. On the whole, acidities of over 2 per cent seem to be more inhibitive to microbial growth than alkaline media of a similar reaction.
5. Streptothrices are favored by an alkaline medium.
6. The size of the colonies of soil bacteria appears to increase as the neutral point is approached from either direction.
7. The growth characteristics and chromogenesis of certain bacteria are much changed when growing on unfavorable media.
8. Some fungi have a remarkable ability of thriving in very acid or alkaline media, the character of the growth of the organism, being as a rule, less subject to variability than is the case of bacteria.

V

Effect on Bacterial Counts of Sterilization of Agar Media at Different Pressures and Temperatures

The question often arises whether the different sterilization pressures obtained in an autoclave, have any influence on the bacterial count obtained from the media so treated. It is the common idea that there is little if any harm done to ordinary agar media by sterilizing in an autoclave, provided the temperature reached is not too high or the period of sterilization process not too long continued. Probably the most common recommendation is to heat the media in an autoclave at 15 pounds pressure for 15 minutes. Of course gelatin, milk or blood serum media must be treated differently, but only agar media are here discussed. Moore (31) states that media sterilized under pressure in an autoclave is detrimental to the growth of certain pathogenic bacteria. Cook (7) found no difference in the counts obtained with media which had been sterilized at flowing steam and by autoclaveing for 15 minutes at 15 pounds pressure. Most bacteriologists agree that long continued heating is injurious either because of chemical changes wrought in the medium or to precipitation of some of the nutrients.

In the following experiment four pressures were used at which to sterilize the media. These are:

(1) Boiling water, i. e. atmospheric pressure.
(2) One atmosphere.
(3) Two atmospheres.
(4) Three atmospheres.

The sterilization in boiling water was accomplished by simply immersing the tubes of media in a vessel containing the hot water for one-half

TABLE X

INFLUENCE OF STERILIZATION OF MEDIA AT VARYING PRESSURES ON BACTERIAL COUNTS[1]

	Medium I				Medium II			
	Boiling Water	1 Atmosphere	2 Atmospheres	3 Atmospheres	Boiling Water	1 Atmosphere	2 Atmospheres	3 Atmospheres
Soil I.....	4.30	3.70	3.65	3.10	6.50	6.10	4.75	2.10
Dil. 1:50,000	4.50	3.90	3.50	3.35	6.85	6.10	4.60	2.00
	4.75	4.00	3.35	4.05	6.45	6.20	5.00	2.30
	5.00	3.50	3.85	3.65	5.85	6.25	4.55	1.95
Average	4.51	3.78	3.59	3.54	6.41	6.16	4.75	2.09
Soil II.....	3.25	2.80	2.90	3.30	6.20	5.85	5.45	3.50
Dil. 1:50,000	3.60	2.50	3.00	3.50	6.25	6.00	5.55	3.25
	3.60	2.70	2.50	3.75	6.05	5.65	4.80	3.50
	3.25	2.85	2.60	3.70	6.60	5.35	4.70	3.20
Average	3.43	2.71	2.75	3.56	6.28	5.71	5.13	3.36
Soil III.....	6.35	5.65	4.55	6.15	7.60	6.40	5.40	2.75
Dil. 1:50,000	6.50	5.25	4.75	6.30	7.45	6.30	5.15	2.40
	6.00	5.20	5.65	5.90	7.10	6.05	5.00	2.20
	6.50	5.40	5.00	5.55	6.50	5.75	4.75	2.20
Average	6.34	5.38	5.24	5.98	7.16	6.13	5.08	2.39
Water III...	550	500	390	395	420	400	325	255
Dil. 1:5	530	540	400	390	400	375	300	250
	495	550	355	425	375	350	315	270
	520	500	350	390	410	400	345	230
Average	524	523	374	400	401	381	321	251

[1] Millions of bacteria per gram of soil for Soils I, II and III. Number of bacteria per c.c. of water for Water III.

hour on two successive days. A 15-minute period was used in sterilizing in the autoclave. Three soils and a sample of water were used as a source of inoculating material. Two media were used, namely, a peptone —KCl agar (I) and, Lipman and Brown's modified synthetic agar (II). All media were treated in precisely the same manner except in the method of sterilization. Table X gives the results obtained in this experiment.

The results are represented graphically in figure 5. Photographs of the plates are given in Plate IV (fig. 2) and Plate V (fig. 1, 2).

Discussion of Data and Curves. Since the results obtained with the two media are quite different each will be discussed in turn. Medium I (peptone) gives in all cases the highest counts, when the sterilization is accomplished by boiling water. This is more marked in the three soils tested than in the water sample, which shows practically no difference in counts between sterilization with hot water or at a pressure of one atmosphere. The average decrease in the number of colonies due to sterilization at one atmosphere is approximately 14 per cent. There is a further decrease at 2 atmospheres, but at 3 atmospheres, strange to say, in three of the four samples tested there is an increase in the number of colonies developing. Such results are difficult to explain satisfactorily unless we consider

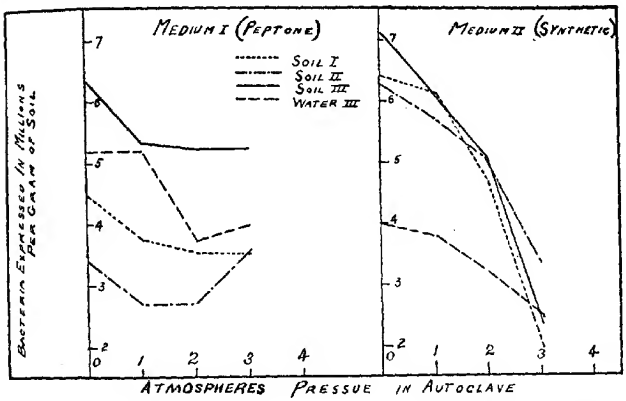

Fig. 5.—Diagram showing the influence of sterilization at different temperatures on bacterial counts.

that peptone is broken down into simpler amino-acids or changed in some way by the high heat obtained at 2 atmospheres pressure in the autoclave, and then on raising this to 3 atmospheres, another favorable change takes place making some of these compounds more available, or else less toxic. From the data at hand it appears that sterilization at 100° C. at atmospheric pressure is preferable for peptone media, but it is possible that if pressures below one atmosphere were used, or if the time were lessened, the pabulum may not be rendered less satisfactory for bacterial development.

Passing on to Medium II (synthetic), it is seen that there is a gradual and almost constant decrease in the bacterial counts as we pass from a sterilization temperature of 100° C. to one much higher which is obtained at a pressure of 3 atmospheres in an autoclave. This seems logical, as it is likely that the composition of the medium becomes considerably

altered at the high temperatures, rendering the nutrients less available or more toxic for bacteria.

Summary. Although these results are by no means conclusive, still the evidence at hand is decidedly in favor of a sterilizing temperature of 100° C. In the experiment this was kept up for 30 minutes on 2 successive days. The sterilization was complete.

The high temperatures produced at pressures of over one atmosphere in an autoclave render the media unfavorable for bacterial growth. This experiment also shows the great superiority of the synthetic over the peptone medium.

VI

Effect of Storage of Media on Bacterial Counts

Much has been said but little done on the question of the deterioration of culture media with age. It is generally supposed that media do deteriorate when stored for a period of time but little data of real value are available. Conn (6) showed that soil extract gelatin may change greatly over night, and considers that under certain conditions agar media may also change in a longer or shorter period of time. Petri and Maassen (32) state that media should be well plugged and stored in the dark, as light and oxygen have an injurious effect on it. Ravenel (36) claims media may be preserved several months if tin-foil is placed over the top of the tubes or flasks. Bioletti (1) recommends using an antiseptic-treated cotton plug covered with a rubber cap. Pierce (33) reports that he finds the method of sealing tubes of media with paraffin of much value in preserving cultures. Hill (19) states that a medium should not be stored in large quantities as it is necessary to sterilize it every time a portion is withdrawn, thus injuring it.

Dipping the plugs of the tubes or flasks of media in paraffin has often been used to prevent evaporation. Also humidors where a saturated atmosphere is preserved have been proposed for storing media.

Many isolated filtration plants and laboratories buy their media in large lots which may be stored for several months. Often in the laboratory it is desirable to know if it is allowable to compare counts from one batch of medium with another batch prepared some time before. Also the question arises as to whether it is proper to use the same batch of medium in a long continued experiment.

To throw light upon some of these points an experiment with two media namely: (I) Peptone—KCl agar and (II) Lipman and Brown's modified synthetic agar, was begun. Medium I was stored in the test tubes and flasks in a cool, dark place, and tested monthly against freshly made media. Tubes both with paraffined plugs and with unparaffined plugs were used. The plugs which were treated with paraffin were cut off close to the mouth of the tube, and dipped into the molten wax. Well

made, close fitting plugs were used to plug the other half of the tubes. The flasks used were of 500 c.c. capacity, each containing about 350 c.c. of medium. In order to make sure that each of these media was uniform throughout, it was all prepared in one large batch, and thoroughly mixed before tubing. Especial care was taken to test the reaction and to adjust it in the case of Medium I, both before and after sterilization.

Tests were made at intervals of about a month for six months. As it was contemplated that if any differences in counts were obtained during the course of this experiment, such would probably be small, ten duplicate plates were poured of *each* medium. The experimental error method of reporting results is used as where so many plates are poured, but little variation is probable, and a good average is possible. The same ingredients precisely were used in the freshly made media as were present in the original batch; even the tap water was stored in a large bottle. Counts were made after 120 hours' incubation at a temperature of 22° to 23° C. The soil and water samples used are from different sources, but of course the same samples were used in the individual tests.

TABLE XI

WEIGHT OF MEDIUM IN TUBES WITH PARAFFINED PLUGS

Wgt. of Medium	Freshly Prepared Oct. 20 gm.	Nov. 16 gm.	Dec. 15 gm.	Jan. 15 gm.	Feb. 18 gm.	Mar. 18 gm.	Apr. 10 gm.
In 10 Tubes....	93.00	91.00	85.40	81.00	78.80	73.00	70.00
In Tube I....	9.89	9.53	8.51	7.75	6.97	6.01	5.83
In Tube II....	9.91	9.85	9.25	8.80	8.31	7.85	7.61
In Tube III[1]...	.....	.....	.....	.....	.....	.....	.....

[1] Tube was broken.

A series of ten paraffined and ten unparaffined tubes were weighed monthly to obtain the loss of moisture by evaporation. Three tubes from each set were also weighed singly for purposes of comparison. Tables XI, XII and XIII show the results obtained.

The acidity of the various media was also tested from time to time. The data thus obtained may be found in Table XIV.

Tables XI, XII and XIII show that there is a great loss of moisture by evaporation on storing media. The tubes with the unparaffined plugs lost nearly twice as much moisture as the tubes with paraffined plugs. In some cases the former suffered a loss in water content of 50 per cent in 6 months. Although the method of saturating the plugs with paraffin aids in hindering evaporation yet the loss is considerable after a few months. In one month the maximum percentage loss of moisture from any of the tubes was 6 per cent. This is not enough to injure the medium seriously as is shown in Table XIV, the rise in acidity being very small. The acidity of the medium of course increases with the evaporation of water. The

nutrients too become more concentrated and possibly changed in nature due to long storage. After 6 months the acidity of the medium in the unparaffined tubes had nearly doubled itself. How this effects the bacterial counts is shown in Table XV. The curves in figure 6 show that the increase in acidity is directly proportional to the loss of moisture from the media.

TABLE XII

WEIGHT OF MEDIUM IN TUBES WITH UNPARAFFINED PLUGS

Wgt. of Medium	Freshly Prepared Oct. 20 gm.	Nov. 16 gm.	Dec. 15 gm.	Jan. 15 gm.	Feb. 18 gm.	Mar. 18 gm.	Apr. 10 gm.
In 10 Tubes....	87.00	82.00	72.00	65.00	61.40	50.50	45.00
In Tube I....	10.00	9.65	8.69	8.10	7.23	6.67	6.18
In Tube II....	10.39	9.32	8.24	7.47	6.64	5.95	5.50
In Tube III....	9.49	9.15	8.12	7.36	6.55	5.84	5.40

TABLE XIII

PERCENTAGE LOSS OF MOISTURE ON STORAGE OF PARAFFINED AND UNPARAFFINED TUBES

Length of Storage	27 Days	56 Days	87 Days	121 Days	150 Days	173 Days
	%	%	%	%	%	%
Loss in 10 paraffined tubes.....	2.2	8.0	12.8	15.2	21.5	24.7
Av. loss of 2 paraffined tubes.....	2.0	10.4	16.5	22.8	30.0	32.0
Loss in 10 unparaffined tubes.....	5.6	17.2	25.3	29.5	42.0	48.2
Av. loss in 2 unparaffined tubes...	6.0	16.0	23.2	33.5	39.3	43.0

TABLE XIV

EFFECT OF STORAGE OF MEDIA ON ITS ACIDITY

Length of Storage	Original Acidity	27 Days	56 Days	121 Days	173 Days
	%	%	%	%	%
Av. acidity of 3 paraffined tubes...	1	1.10	1.24	1.42	1.62
Av. acidity of 3 unparaffined tubes.	1	1.13	1.38	1.61	1.86
Medium I stored in flasks.....	1	....	....	1.20	....
Medium II stored in flasks.....	0.4	....	....	0.60	....

Each figure in Table XV represents ten plates except in Water Sample III and in column 7, where the averages are the results of five plates only. The experimental error was calculated from the formula

$$E = \pm .6745\sqrt{\frac{\Sigma D^2}{n(n-1)}}$$

E = Probable error.

D = Deviation from the average.

n = Number of determinations.

Discussion of Tables XV and XVI and Curves. The foregoing data show that the media which were used in this experiment deteriorate if stored for any great length of time. If the plugs of the tubes are not paraffined the medium becomes poor very rapidly. With a peptone agar, in one month a percentage decrease in bacterial counts over the check of 26.8 per cent was noted. Such a difference between two media is enough to make worthless any quantitative work in which they are used. This de-

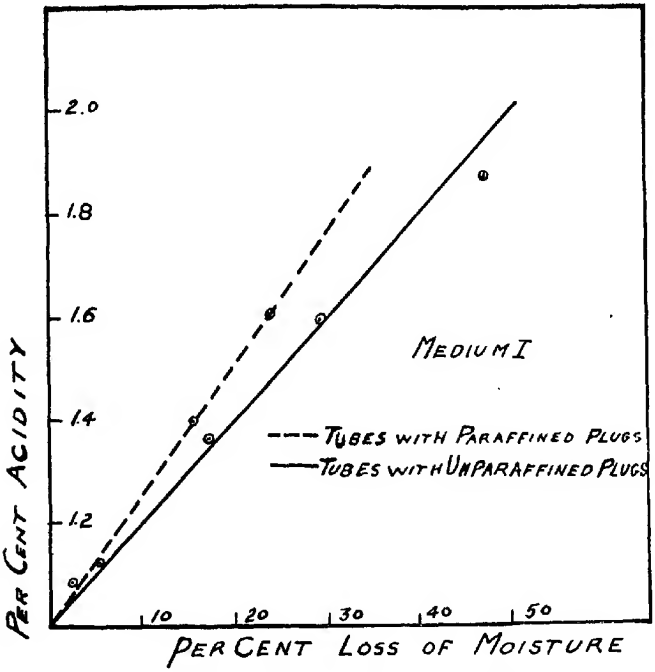

Fig. 6.—Diagram showing the correlation of moisture loss and acidity increase.

terioration becomes greater the longer the time of storage. The curves in figure 7 show that there is apparently a direct ratio between time of storage and bacterial counts.

Dipping the plug end of the tubes in molten paraffin helped to preserve the media to some extent. After two month's storage the percentage decrease in bacterial count was only 12.7, but for longer periods it did not seem to hinder evaporation entirely. The evaporation is approximately one-half that of the tubes with plain cotton plugs.

Flasked media keep better than tubed media, but even here there was a noticeable deterioration after four months. Had the flasks been sealed with paraffin probably the medium would have been preserved much

longer. Up to three months there is little change in the bacterial counts on peptone agar stored in flasks.

TABLE XV

EFFECT OF STORAGE OF MEDIA ON BACTERIAL COUNTS[1]

Days storage	Material tested	Check Medium I freshly prepared	Medium I Plugs paraffined	Medium I Plugs not paraffined	Medium I Flasks	Medium I. Not paraffined + H_2O
27	Soil V	11.25±.37	8.75±.80	8.31±.13	.....	.....
	Soil VI	15.75±.32	15.20±.95	11.30±.41	.....	.....
	Water III	310±40	310±20	230±30	.....	.....
56	Soil I	1.70±.05	1.40±.05	1.05±.04	1.70±.10	.....
	Soil II	1.85±.09	1.60±.04	1.10±.06	2.05±.13	.....
	Water III	1100±40	1020±60	800±15	1060±30	.....
87	Soil I	1.37±.15	.90±.08	.55±.014	1.24±.10	1.10±.11
	Soil II	3.05±.12	1.52±.02	.97±.02	2.27±.07	2.65±.18
	Water III	590±40	500±27	190±15	740±25	.....
121	Soil I	3.15±.065	1.95±.05	1.335±.12	1.91±.19	1.70±.12
	Soil II	3.25±.075	2.20±.08	1.75±.15	2.05±.15	.....
	Water III	320±16	260±15	200± 8	270±20	.....
150	Soil II	3.30±.13	2.00±.09	1.30±.10	2.90±.20	2.60±.13
	Soil III	7.30±.25	4.80±.21	2.60±.12	5.70±.19	6.1±.22
173	Soil I	2.61±.20	1.30±.09	0.79±.06	.....	Precipit'ed
	Soil II	3.21±.16	1.80±.11	0.92±.09	.....	.....

[1] About 4 c.c. sterile H_2O was added to each tube before melting the medium. Bacteria are expressed in millions per gram of soil.

Since deterioration, evaporation and acidity increases are proportional to the length of storage, and since it has been shown earlier in this paper

TABLE XVI

EFFECT OF STORAGE OF MEDIA ON BACTERIAL COUNTS
Average per cent decrease in bacterial counts over check

Days Storage	Medium I Paraffined plugs	Medium I Plugs not paraffined	Medium I 500-c.c. flasks	Medium I. Plugs not paraffined + 4c.c. H_2O[1]
27	7.9	26.8	0	..
56	12.7	35.2	0.5	..
87	33.2	66.0	5.0	14
121	30.0	53.3	1.37	46
150	36.5	62.0	17.0	18
170	47.0	71.0	....	..

[1] Four c.c. sterile H_2O were added to each tube before melting the medium.

that reaction exerts a tremendous influence on colony development, it seems reasonable to conclude that acidity is here the primary limiting fac-

tor, with perhaps the high concentration of the nutrients as a secondary inhibiting factor for bacterial growth.

The custom of sometimes adding sterile water to tubes of dried-out agar media furnished grounds for testing out this point in a partial way.

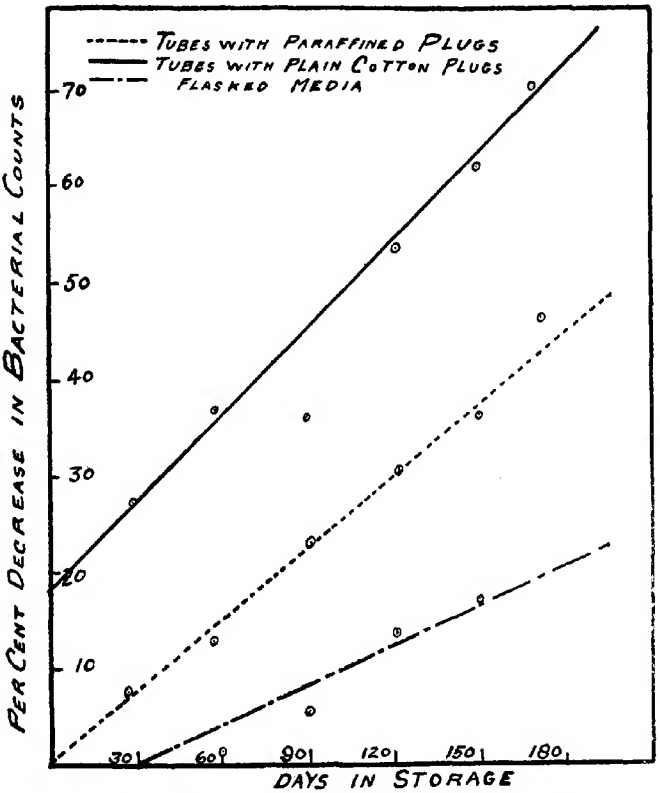

Fig. 7.—Diagram showing the deterioration of media on storage.

The results obtained show that an addition of water aids in restoring the good qualities of the medium, but the restoration is not complete; besides, very often a flocculent precipitate is obtained. The method cannot be recommended except in rough work.

General Summary

After briefly reviewing the literature touching on the subject it has been pointed out that:

1. Although there are present in agar considerable amounts of soluble nutrients including nitrogenous materials, these are available for microorganisms only to a very limited extent. If the conditions are made favorable however, microbial activity becomes well marked, one of the results being a transformation of a part of the nitrogenous matter into ammonia.

2. The "purified" agar obtained by washing the crude product with dilute acid, and subsequent precipitation of a water solution of the agar by means of alcohol or acetone, is not injured as regards its use as a substratum, since it is firm and does not reduce the bacterial counts obtained from soil, when compared with ordinary agar.

3. A concentration of agar of between 1.25 to 1.5 per cent has been found to be most favorable with the media used, and with soil as the inoculum. Low concentrations of agar are of value in the study of certain microörganisms due to the large colony development. High concentrations are useful where either slow growing organisms are to be cultivated or where the medium is to be stored a long time.

4. Under the conditions of the experiment the optimum reaction of the media tested for the development of maximum numbers of soil or water bacteria, is one between the neutral point and an acidity of $+0.5$ per cent HCl.

5. For soil fungi the optimum acidity is from $+1$ to $+1.5$ per cent HCl.

6. Many soil bacteria and fungi are able to develop colonies at comparatively high concentrations of hydrogen or hydroxyl ions.

7. Streptothrices are favored by an alkaline medium.

8. The size of the colonies of soil bacteria appears to increase as the neutral point is approached from either direction.

9. The growth characteristics and chromogenesis of certain bacteria are much changed when growing on an unfavorable medium.

10. Some fungi have a remarkable ability of being able to thrive in very acid or alkaline media, the character of the growth of the organism being as a rule less subject to variability than is the case with bacteria.

11. With both peptone and "synthetic" agar, the maximum counts were obtained when the media were sterilized in boiling water for one-half hour on two successive days. Sterilization in the autoclave at a pressure of one atmosphere for 15 minutes did not injure the medium to a very great extent, but at higher temperatures and pressures, a serious deleterious effect was noted.

12. Lipman and Brown's modified synthetic agar (Medium II) gives much higher counts than peptone—KCl agar (Medium I).

13. Peptone media in tubes (Medium I) deteriorates rapidly on storage. This is due to an increase in acidity caused by the evaporation of water from the tubes and consequent concentration of the agar and nutrients. The loss of water by evaporation, the increase in acidity and the decrease in bacterial colonies developing in the medium, are apparently proportional to the length of the storage period.

14. If the plugs are paraffined, the deterioration is not so rapid. Also if the medium is stored in flasks, it may be kept several months without appreciable change.

15. Addition of water to tubes of dried-out media does not restore entirely the original properties of the media, but it aids in correcting the acidity, and hence makes the substratum more favorable for bacterial growth.

16. Synthetic agar (Medium II) also appears to be injured on keeping in storage, but the extent of this injury has not been determined.

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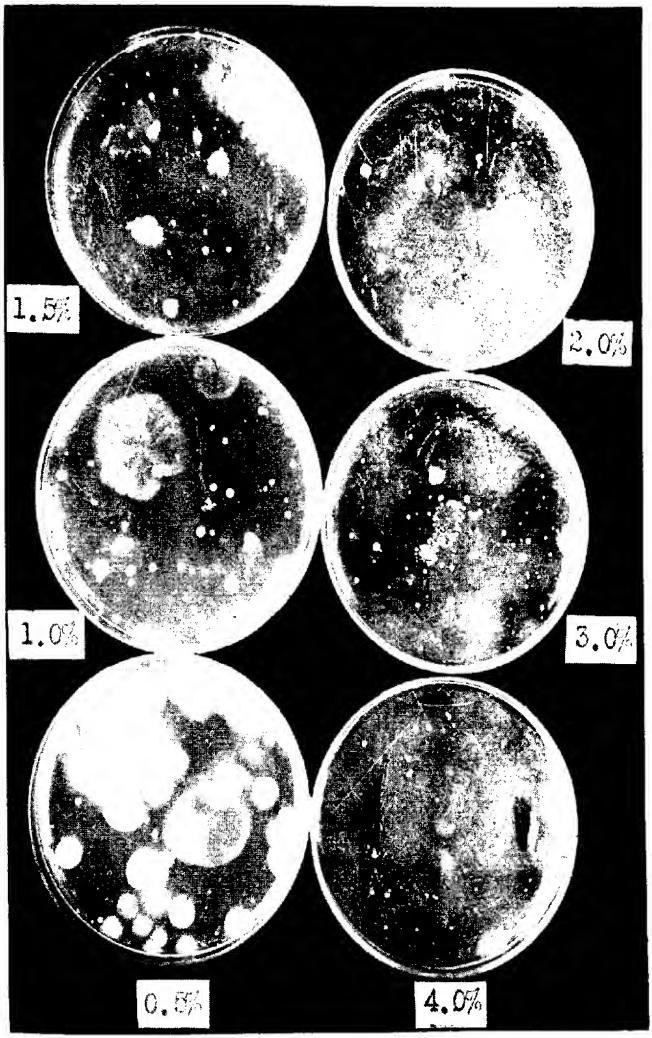
PLATE I

Plates showing the effect of concentration of agar in media on bacterial counts, Soil I.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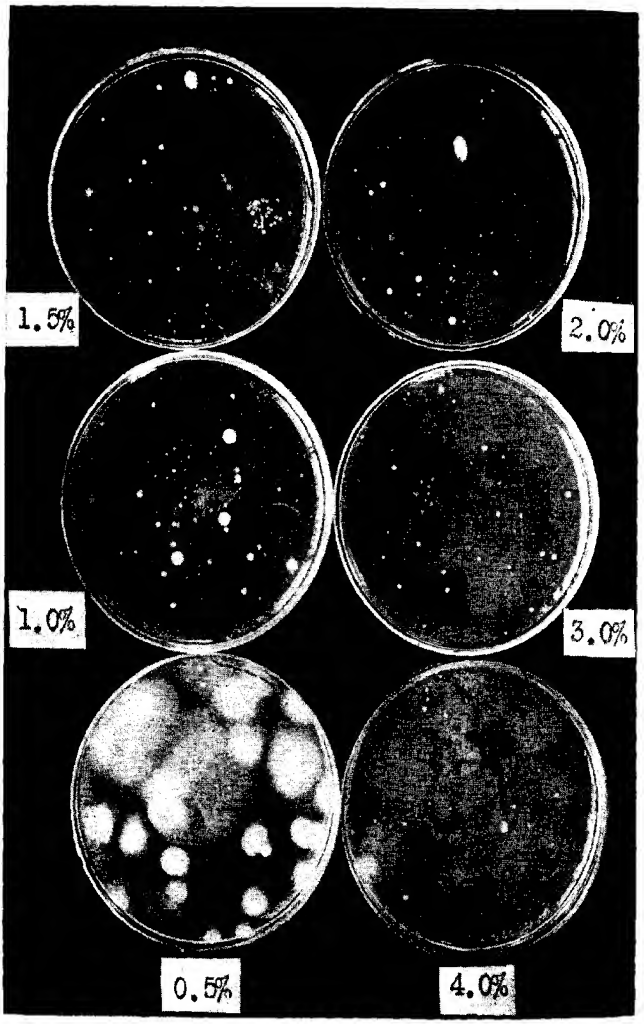
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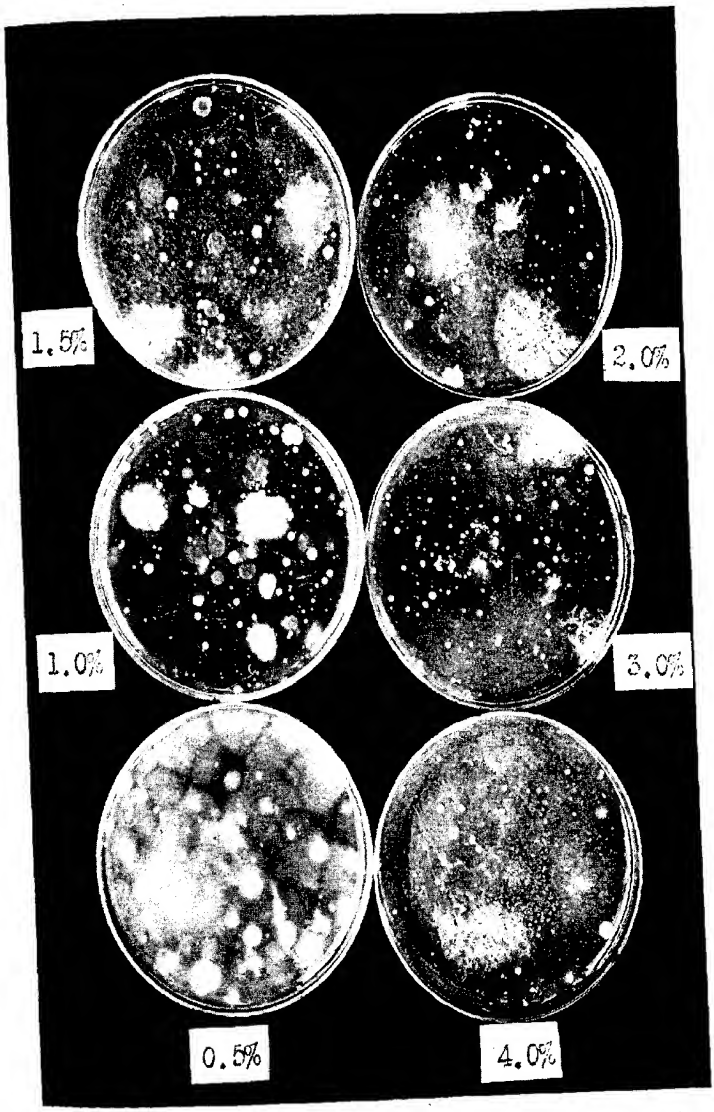
PLATE II

Plates showing the effect of concentration of agar in media on bacterial counts, Soil II.

PLATE III

Plates showing the effect of concentration of agar in media on bacterial counts, Soil IV.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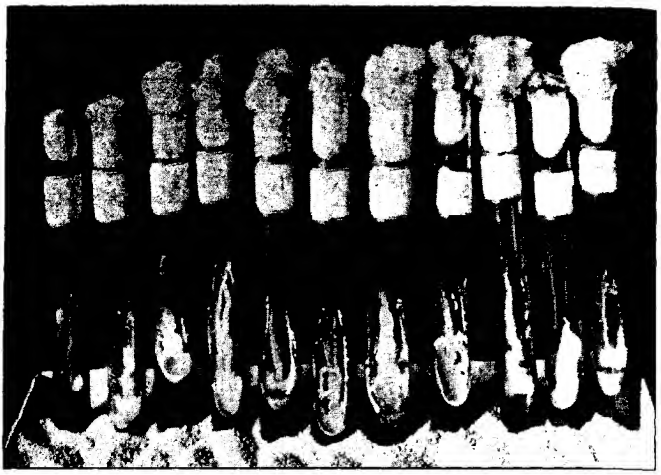

Fig. 1

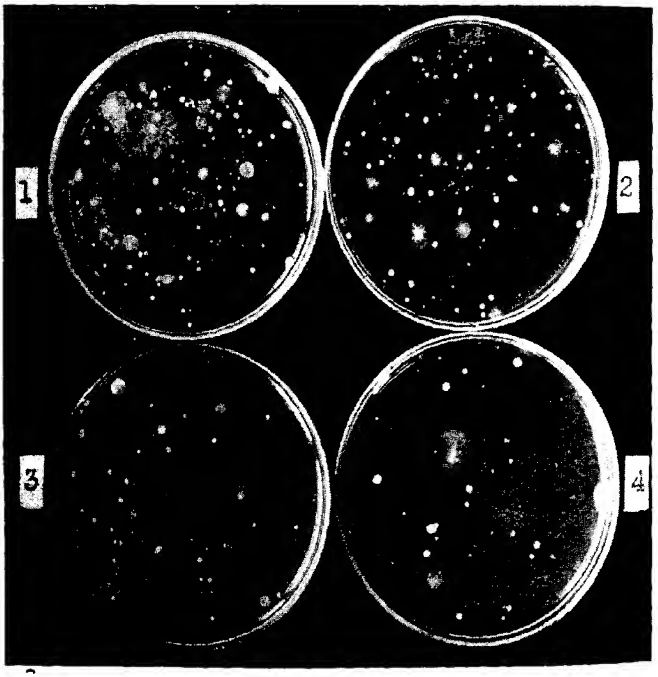

Fig. 2

PLATE IV

Fig. 1.—Growth of *B. subtilis* on media of different reactions.

Fig. 2.—Plates showing the effect of sterilization of media at different pressures on bacterial counts, Soil I, Medium II, (Synthetic).

No. 1, Boiling Water.

No. 2, 1 Atmosphere.

No. 3, 2 Atmospheres.

No. 4, 3 Atmospheres.

PLATE V

Plates showing the effect of sterilization of media at different pressures on bacterial counts. Fig. 1.—Water III, Medium I (Peptone).

No. 1, Boiling Water.
No. 2, 1 Atmosphere.
No. 3, 2 Atmospheres.
No. 4, 3 Atmospheres.

Fig. 2.—Water III, Medium II (Synthetic).

No. 1, Boiling Water.
No. 2, 1 Atmosphere.
No. 3, 2 Atmospheres.
No. 4, 3 Atmospheres.

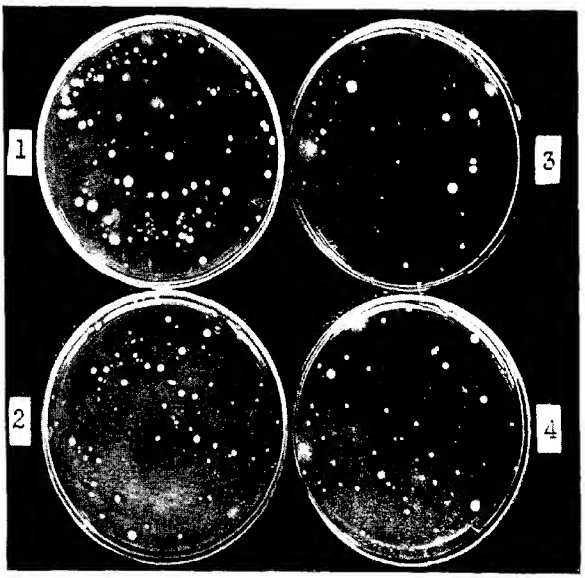

Fig. 1

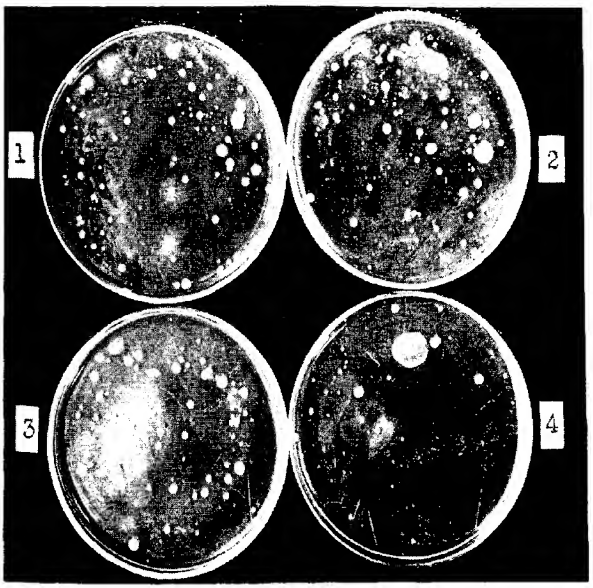

Fig. 2

THE ORGANIC PHOSPHORUS OF SOIL[1]

By

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Introduction

That there must be phosphorus in organic combination in soil is universally recognized. It is shown in the historical section of this paper that there has been no method which can be said to give even an approximation as to the actual amount of organic phosphorus in soils. That such a method would be desirable is obvious. Phosphorus, from an economic standpoint, is perhaps the most important plant-food element in a consideration of the fertility of most of the soils of the United States. In many cases it is actually deficient in amount, and in many other cases, while present in fairly high proportions, it is in such condition that it is unavailable to plants. The presence of large amounts of organic matter in soils is known in many instances to change inert phosphates to such a condition that plants may utilize them. This is usually attributed to the increase in bacterial action due to the organic matter. As to just why this should make inert phosphates available there is some doubt, but the general opinion is that carbonic and other acids dissolve the phosphates. We would not venture to say whether or not this is the predominant action of the organic matter. It may be also that phosphorus actually taken up into the bacterial cells or into molds is of great importance, thus rendering available inert phosphates. It has been shown by Stoklasa (17), for instance, that some of the common soil bacterial cells contain about 1.5 per cent of phosphorus.

There is very little known as to the kinds of organic phosphorus compounds present in soil. However, from a knowledge of the organic materials added to soils one can draw some conclusions, but even here data are insufficient or unreliable. For instance, there is very little known in regard to the exact composition of vegetable material in organic phosphorus compounds. Phytin is known to be present to the extent of roughly 50 per cent of the phosphorus in the seeds of many grains, beans, etc. (5, p. 52), but as to the phosphorus in the stems and leaves very little is known. Practically nothing is known as to the quantitative relations of the phosphorus compounds in corn stover, oat straw, manures and such material which make up a large part of the organic matter ultimately finding its way to soil. The knowledge as to the quantitative composition of vegetable material in regard to its composition of nucleic

[1] Received for publication August 7, 1916.

acid is negligible, yet since all cellular material contains nucleoproteins, this class of compounds must be added to soils in relatively large amounts.

The true lecithins are not found in plants (5, p. 73), and the phosphatids of the vegetable and animal kingdoms are essentially different. Not much is known in regard to plant phosphatids, but they seem to be widely but sparingly distributed. Because the large phosphatid molecule contains only one phosphorus atom, it is not probable that the phosphorus combined in phosphatids in soils is quantitatively of much importance.

Without going into the rather extensive literature on the chemical constitution of bacteria, molds and yeasts at this time, nucleoproteins, phosphatids and phytin are known to be present in many of them (16).

There is some doubt as to whether phosphoproteins are present in vegetable material at all. While some have apparently been isolated, yet it is claimed by many investigators that the phosphorus is present as an impurity (5, p. 48).

To generalize broadly from the rather meager data available, nucleoproteins and phytin probably make up most of the organic phosphorus compounds added to soil, with phosphatids next, and a very small quantity of phosphoproteins. As to the fate of the phosphorus compounds upon entering the soil, little can be said. So far as we know, nothing in regard to the action of soil bacteria upon phytin is known. It is known, however, to be very readily decomposed by enzymatic action (1). Stoklasa (17) has shown that nucleic acid is perhaps less readily attacked by bacteria than phosphatids. Casein, a phosphoprotein, is known to be readily decomposed upon being added to soil.

Historical

A brief review of the work done upon the organic phosphorus of the soil will be given here. For a quite complete bibliography on the subject up to the year 1909 the reader is referred to the article by Stewart (16).

Grandeau (9) believed that the phosphorus associated with the *matière noire* was in organic combination, and this view was upheld by other soil investigators. Others have opposed this idea. For instance, Van Bemmelen (18) believed that all of the phosphorus in the *matière noire* was simply absorbed. No direct or positive work tending to prove or disprove the question appeared until Fraps (6, 7) attacked the problem. He showed, amongst other things, that minerals, present in most soils, such at apatite, variscite, wavelite, vivianite, and many others, are somewhat soluble in 4 per cent ammonia. It was also found by him that ammonia dissolved considerable phosphorus from ignited soils and that the amount of phosphorus dissolved from ignited soils was increased by previous treatment with dilute acid. His conclusion was that the phosphorus dissolved by ammonia is certainly not all organic.

Stewart (16) determined the per cent of *matière noire* precipitated by 1 per cent hydrochloric acid and also the per cent of carbon, nitrogen

and phosphorus so precipitated. These determinations were carried out on both untreated and 1 per cent acid extracted soil. From the fact that 238 pounds more phosphorus was obtained in the ammonia extract of the acid treated soil than in the untreated soil, Stewart concludes that all of this 238 pounds must be in organic combination; but Fraps' experiments showing that acid extracted ignited soils, necessarily containing no organic phosphorus, give more phosphorus into solution with ammonia than the unextracted ignited soils, render Stewart's conclusion, to say the least, not absolute. Since only 55 pounds of phosphorus was precipitated from the ammoniacal solution of the *matière noire* by acid and since less than 50 per cent of the carbon and nitrogen was so precipitated, Stewart concludes that there is considerable organic phosphorus in the filtrate. He is no doubt right, but as pointed out again by Fraps, his conclusion does not follow from the premises.

Stewart extracted his soil with cold 12 per cent hydrochloric acid. After washing free from chlorides, one portion was re-extracted with 12 per cent hydrochloric acid which dissolved 95 pounds of phosphous per acre. Another portion was extracted with 4 per cent ammonia which dissolved 555 pounds of phosphorus per acre. The difference, 460, must represent, according to Stewart, organic phosphorus, but Fraps' finding that ammonia dissolves considerable phosphorus from minerals previously treated with 12 per cent hydrochloric acid makes this method of Stewart unreliable.

Schmoeger (13) found that ignition increased the phosphorus dissolved by 12 per cent hydrochloric acid, and from this fact assumed that the increased phosphorus dissolved after ignition was organic. In view of work already cited this can no longer be considered even approximately correct.

Hopkins and Pettit (10) proposed a calculation method to estimate the organic phosphorus of soil. In soils having the surface and subsoil with the same potassium content, the mineral composition of the two layers is assumed to be the same. Therefore, the excess of phosphorus in the surface soil is assumed to be organic. Such a method carries with it too many broad assumptions to be of much accuracy. Stewart found that fairly close agreement was obtained on one soil between this calculation method, the phosphorus dissolved by ammonia, the increase in phosphorus dissolved by 12 per cent hydrochloric acid after ignition of soil, and the increase of phosphorus dissolved after subjecting the soil to heat in the autoclave at 140° to 145° C. for 12 hours. The methods in general, however, have not been found to give concordant results. In view of the findings, particularly of Fraps, and also when we consider the assumptions necessarily involved in all of the methods, none of them can be considered to give accurate results.

There have been some successful attempts to isolate organic phosphorus compounds from the soil. Aso (2) extracted a soil with alcohol

and ether and obtained a slight residue upon evaporation of the extract which was analyzed for phosphorus. According to the figures so obtained, he calculated his soil to contain 0.049 per cent lecithin. Stoklasa (17) also found indications of a small amount of lecithin by the same method. He was unable to isolate nucleic acid from soils. Shorey (14) isolated nucleic acid from a number of soils. No attempt was made by him to obtain quantitative results. From his observations, however, he states that nucleic acids "form a very appreciable portion of the organic matter of soils."

Experimental

The method which we have developed is not, for the present at least, proposed as giving the total organic phosphorus of soils, but rather as the total organic phosphorus in the dilute alkali extract of soils. This, of course, would be the lower limit for organic phosphorus. It has previously been shown in a publication from this laboratory that dilute sodium hydroxide extracts nucleic acid completely from soils (12). Nucleic acid is perhaps the most abundant organic phosphorus compound of soil and it may be that dilute alkali dissolves nearly all of the organic phosphorus. Confirmation of this will have to await further investigation.

The method which has been adopted after much experimentation is a combination of the Forbes (4) magnesia mixture method and the Emmet-Grindley (3) method, both slightly modified. The method in detail is as follows:

The soil extract, however obtained, is made slightly ammoniacal, sufficient ammonium chloride added to give a 5 to 10 per cent solution of the salt, and then magnesia added at the rate of 20 c.c. per 100 c.c. of solution. The magnesia mixture is added from a burette, a drop at a time, with constant stirring. The solution is allowed to stand 3 hours. The Forbes method calls for a much more strongly alkaline solution and a period of 12 hours of standing before filtration. It has been shown by Gooch and Austin (8) that the precipitation of a soluble phosphate is quantitative in the presence of a slight excess of ammonia if a sufficiently large excess of magnesia mixture is present. The reason for adopting the above procedure was to avoid as much hydrolysis of the organic phosphorus compounds as was possible. After standing the three hours, as much of the supernatant clear liquid was withdrawn as was possible. This was filtered. The residual liquid containing the large bulk of the precipitate was transferred to 100-c.c. tubes and centrifuged. The clear supernatant liquid was siphoned from the tubes and passed through the filter paper. The precipitate was washed four times with water containing a trace of magnesia mixture. After washing, the precipitate was collected with about 150 c.c. of water, and 8 c.c. of nitric acid of specific gravity of 1.20 was added for each 100 c.c. of the solution. The mixture was filtered and the residue washed with very dilute nitric acid. The

filtrate and wash water was neutralized with ammonia and concentrated under diminished pressure to 125 c.c. The solution was then heated to 60° C. First, from 5 to 8 c.c. of nitric acid of specific gravity of 1.20, and then 100 c.c. of clear neutral ammonium molybdate were added. The temperature was maintained at 60° C. for 15 minutes and the solution stirred. The solution was then removed from the bath, and after standing about 2 hours the precipitate was filtered off and washed as usual. It was then dissolved in 200 c.c. of 2 per cent ammonia, 20 gm. of ammonium chloride added and 50 c.c. of magnesia mixture. After standing at least 3 hours the precipitate was filtered and washed in the usual manner and then dissolved in nitric acid and evaporated to complete dryness to remove silica. From this point the phosphorus was determined according to the method of Lorenz (11).

The total phosphorus of the soil was determined by the Lorenz method, the magnesium nitrate method of ignition being used. The total phosphorus of the various extracts was determined by the same method. In all cases the extracts were evaporated to dryness with magnesium nitrate solution and then ignited.

First of all, the proportion of the phosphorous extracted by N/5 acid in organic combination was determined. One hundred fifty grams of a silt loam soil were placed in each of 4 bottles and 600 c.c. of hydrochloric acid of such a strength that after contact with the soil it was N/5, were added to each bottle. After shaking 1 hour the mixtures were filtered. Two 400-c.c. portions of the clear filtrate were analyzed for total phosphorus and two 400-c.c. portions were analyzed for inorganic phosphates exactly as indicated above. The results follow:

TABLE I

COMPOSITION OF THE PHOSPHORUS IN THE N/5—HCl EXTRACT, EXPRESSED IN PER CENT OF SOIL

Total P in Soil	Total P in N/5—HCl Extract	Inorganic P in N/5—HCl Extract
0.0345	0.0112	0.01118

Therefore it is seen that all of the phosphorus extracted from this soil by dilute acid is inorganic in nature.

Two per cent ammonia was next experimented with. Because of the well-known fact that previous extraction of soils by dilute acids renders more of the organic matter soluble in dilute ammonia, and because of the fact that, as shown above, the dilute acid extracts no organic phosphorus, it was decided to extract the soil first with acid. The method used was the usual one, namely, placing about 150 gm. of the well ground soil on a Büchner funnel over filter paper and with gentle suction applied, about 1 per cent hydrochloric acid was poured over the soil in small portions.

The soil was then washed thoroughly. In no case was the acid allowed to remain in contact with the soil for more than an hour.

After air-drying, the 2 per cent ammonia extract was made by shaking a mixture of 1 part of soil to 4 parts of alkali for 1 hour and then centrifuging for 5 minutes in the machine used for this work (15) in this laboratory. That 5 minutes' whirling in the machine takes out all phosphorus compounds in suspension is shown by the following experiment.

An alkali and soil mixture prepared in the usual way was placed in the centrifuge and after whirling 5 minutes, 50 c.c. were withdrawn and analyzed for total phosphorus. The whirling of the machine was continued for 10 minutes more and a second portion of 50 c.c. withdrawn and analyzed for total phosphorus. After whirling 45 minutes more, the same operation was repeated. The results are given in Table II.

TABLE II

RESULTS SHOWING THE EFFICIENCY OF THE CLARIFICATION OF THE EXTRACT

Time of Whirling in Minutes	Per cent of P in the Extract
5	0.01660
15	0.01657
60	0.01680

In order to test the method for the determination of the inorganic phosphorus in the alkali extract in regard to the completeness of the recovery of inorganic phosphorus, four portions of 200 c.c. of the 2 per cent ammonia extract were prepared in the usual way. To two of the solutions a known volume of a solution of potassium phosphate was added. All of the solutions were then carried through the procedure for the analysis of inorganic phosporus. At the same time the strength of the solution of potassium phosphate was determined by analyzing according to the Lorenz method. Several attempts were made before more than about 94 per cent of the added phosphorus could be determined. Finally by refining the procedure at various points by better washing, etc., a 98.9 per cent recovery was effected.

With the method thus developed, soil from a series of plots at the Experiment Station were analyzed. These plots are located on the Wisconsin drift soil area and are 1/20 acre in size. They have been fallowed since 1908. There are 14 in all, being numbered from 101 to 114. Not all were analyzed. In considering the results the topography of the plot must be taken into consideration. The highest point is near the south end of Plot 110. There is a gentle fan-like slope in all directions, the lowest part being in Plats 101 and 102. The results, together with the results on the soil used in the preliminary tests are given in Table III. This soil is from one of the orchards of the station and is considerably lower in organic matter than the soil from the plots.

The results given in Table III are interesting. It is seen that the two plots receiving the more inert organic matter, namely 102 and 108, are lowest in organic phosphorus. If this is more than accidental, we are unable to assign any significance to the fact. All of the plot soils are higher in organic matter than the orchard soil and it is seen that all are consider-

TABLE III

RESULTS OF THE PHOSPHORUS DETERMINATIONS
(Expressed as per cent of soil)

Soil	Treatment per Acre	Per cent P in Soil	Per cent P in NH_3 Extract	Per cent Inorganic P in NH_3 Extract	Per cent Organic P	Per cent P in NH_3 Extract	Per cent Inorganic P in NH_3 Extract	Per cent Organic P
Orch'd		0.0345	0.0166	0.0075	0.0091	48.1	21.7	26.4
102	2.8 T. Peat annually	0.0608	0.0383	0.0077	0.0206	63.0	12.7	33.9
103	8 T. Manure in 1909 and 1913	0.0451	0.0294	0.0119	0.0175	65.2	26.4	38.8
104	8 T. Clover in 1909 and 1913	0.0490	0.0303	0.0093	0.0210	61.5	19.0	42.9
107	Nothing	0.0459	0.0291	0.0114	0.0217	63.4	24.8	38.8
108	2 T. Oat Straw annually.....	0.0540	0.0327	0.0169	0.0158	60.6	31.3	29.1
111	4 T. Clover annually	0.0518	0.0318	0.0098	0.0220	61.4	18.9	41.2
114	4 T. Manure annually	0.0649	0.0377	0.0095	0.0282	58.1	14.7	43.5

ably higher in organic phosphorus. Before any more definite conclusions are drawn the accumulation of more data must be awaited.

Summary and Conclusions

The data obtained so far can only be regarded as preliminary. The work will be continued along the lines of an investigation into the completeness of the extraction of the organic phosphorus from the soil. Also, such questions as the influence of various factors on the change of organic phosphorus content of soil will be attacked. The availability of organic phosphorus compounds for plants under various treatments will be investigated.

While the method cannot be said to give an absolute knowledge of the organic phosphorus content of soil, it at least gives comparative results and should be of undoubted value in a comparison of various treatments on a single soil or closely related soils.

As a result of the work done so far, it can be definitely stated that a large part of the phosphorus of the soil is organic in nature.

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DOES VANADIUM INTERFERE WITH THE DETERMINATION OF PHOSPHORUS IN SOILS WHEN THE PHOSPHORUS IS WEIGHED AS MAGNESIUM PYROPHOSPHATE?[1]

By

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In a recent paper, Robinson (8) gives the results of the following experiment: "Solutions containing 0.0050 gm. V_2O_5 and 0.00864 gm. P_2O_5 were treated with magnesia mixture in the usual manner and allowed to stand 6 hours; 0.0088 gm. P_2O_5 was found. When the precipitation for the same amounts had stood 17 hours there was apparently 0.0145 gm. P_2O_5. From these experiments it appears that with the small amounts of vanadium and phosphorous occurring in soils a satisfactory separation can be made with magnesia mixture, *provided the precipitate does not stand more than 3 to 4 hours.*"[2] Robinson does not suggest it, but the fact remains that this statement of his calls into question practically all of the determinations of phosphoric acid that have been made on either rocks or soils. Washington (9, p. 165) states that the magnesia precipitate should stand for 12 hours. Hilgard (3) recommends that the precipitate stand 24 hours before filtering. The Halle Experiment Station (10) allows 48 hours for the complete separation of the precipitate.

[2] Our italics.

This list might be continued almost indefinitely, for at least 50 per cent of the texts on quantitative analysis specify that it is desirable that the precipitate stand "over night" and, even in those texts which state that 3 to 4 hours' standing causes complete precipitation, there is no hint that a longer standing may produce erroneous results.

Inasmuch as vanadium is probably present in most soils (7) and rocks (4) in amounts which in some instances equal or even exceed the phosphorous content, it was deemed advisable to see if Robinson's conclusion as to the possible contamination of the magnesium pyro-phosphate by vanadium was actually justifiable.

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A contamination of the magnesium precipitate by vanadium would occur only providing the vanadium was precipitated both by the molybdate and by the magnesia mixture. It is well established that vanadium is precipitated by ammonium molybdate only in the presence of phosphoric acid, and that *complete* precipitation of the vanadium requires a large excess of phosphorous. Cain and Hostetter (1, p. 254) state that 10 mg. of phosphorous is necessary for the complete precipitation of 1 mg. of vanadium.

It would seem, therefore, that in a soil analysis a separation of phosphorous and vanadium would be made, to a large extent at least, in the molybdate precipitation, and that sufficient quantities of vanadium would not remain to contaminate seriously the magnesia precipitate, even if all of the remaining vanadium should be precipitated by the magnesia mixture. On this latter point, Robinson's experiment furnishes the only available data that we have been able to find.

We have, therefore, investigated the behavior of vanadium when added directly to a phosphate solution and precipitated by magnesia mixture as well as when added to soil extracts and precipitated by ammonium molybdate followed by the usual magnesia precipitation.

Experimental

Precipitation of a mixed solution of phosphate and vanadate by magnesia mixture

It will be noted in Robinson's experiment that at the end of 6 hours the P_2O_5 recovered was but 0.00014 gm. in excess of that added, while at the end of 17 hours it was 0.00586 gm. in excess, or slightly more than would be produced by the complete precipitation of the added vanadium. Such a differential rate of precipitation is certainly unusual, and if *complete* precipitation of vanadium occurs in 17 hours it should take more than a single determination to show that only a mere trace is precipitated in 6 hours.

It was suggested to us that a possible explanation of the vanadium precipitation might be found in the well known fact that a high concentration of ammonium salts causes ammonium meta-vanadate to precipitate from solution. We have, therefore, tested the behavior of a mixture of sodium phosphate and ammonium meta-vanadate when treated with magnesia mixture in the presence of varying concentrations of ammonium hydroxide. The volume of the solution in which the precipitation was made was, in each instance, 100 c.c. The data are shown in Table I.

It will be observed that an increase in the weight of the magnesium precipitate was obtained only when the concentration of the ammonia exceeded 20 per cent by volume. In all instances where vanadium was added, the magnesium pyrophosphate was more or less colored, ranging

from light yellow to almost orange. However, the amount of color present did not correspond accurately to increases in weight.

These experiments would indicate that, even if all of the vanadium of soils were present in the ammonium phospho-molybdate precipitate, a contamination of the pyrophosphate precipitate would not normally take place, inasmuch as the concentration of ammonia in the solution should not exceed 20 per cent by volume. There is a possibility, however, that

TABLE I

THE WEIGHTS OF MAGNESIUM PYROPHOSPHATE OBTAINED BY PRECIPITATING PHOSPHORIC ACID WITH MAGNESIA MIXTURE IN THE PRESENCE OF DIFFERENT AMOUNTS OF AMMONIUM META-VANADATE AND VARYING CONCENTRATIONS OF AMMONIA, THE PRECIPITATE IN ALL CASES STANDING 20 HOURS

P_2O_5 Taken gm.	V_2O_5 Taken gm.	$Mg_2P_2O_7$ Found gm.	$Mg_2P_2O_7$ Found gm.	$Mg_2P_2O_7$ Found gm.
		10%[1] NH_4OH	20%[1] NH_4OH	30%[1] NH_4OH
0.0032	None	0.0049	0.0051	0.0050
0.0032	None	0.0050	0.0048	0.0051
0.0032	0.0014	0.0050	0.0049	0.0055
0.0032	0.0014	0.0050	0.0052	0.0061
0.0032	0.0028	0.0050	0.0050	0.0058
0.0032	0.0028	0.0050	0.0051	0.0066
0.0032	0.0042	0.0051	.....	.....
0.0032	0.0042	0.0051	.....	.....

[1] Per cent by volume of concentrated ammonia.

the vanadium might be precipitated from solution by some conditions which did not occur in our experiments, and we have, therefore, tested the effect of vanadium on the determination of the phosphorous contained in a soil solution.

The effect of vanadium on the determination of soil phosphorous

A standard solution of ammonium meta-vanadate was prepared and added in different amounts to a soil solution prepared by a 5-day digestion of a soil with 1.115 specific gravity hydrochloric acid and subsequent procedure as for Solution "A" in Hilgard's (3) manual. The hydrochloric acid solution after the addition of the vanadium was evaporated with nitric acid to change chlorides into nitrates, taken up with dilute nitric acid and the phosphoric acid precipitated with ammonium molybdate and ammonium nitrate in the usual manner. In all cases the molybdate precipitate was left in a warm place over night. The influence of the vanadium could be readily observed, since where vanadium was added the precipitate was colored more or less deeply orange. The magnesium ammonium phosphate was precipitated as usual, in an ammonia concentration of 15 to 18 per cent by volume, and after standing for 3 hours was filtered off and weighed. The filtrates were then allowed to stand 24 hours longer in order to determine how much V_2O_5 would be precipi-

tated between 3 hours and 27 hours. According to Robinson's data, all of the vanadium precipitated by the ammonium phospho-molybdate should have appeared here. Table II shows the results obtained.

TABLE II

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS, MAGNESIUM PRECIPITATE REMOVED AFTER 3 HOURS

P_2O_5 Taken gm.	V_2O_5 Added gm.	$Mg_2P_2O_7$, 3 hrs. gm.	P_2O_5, 3 hrs. gm.	$Mg_2P_2O_7$ in filtrate, 3 to 27 hrs. gm.
None	0.0028	None	None	0.0001
None	0.0028	0.0002	.....	0.0002
0.0026	None	0.0044	0.0028	0.0001
0.0026	None	0.0041	0.0026	None
0.0026	None	0.0043	0.0027	0.0001
0.0026	0.0014	0.0037	0.0024	.....
0.0026	0.0014	0.0041	0.0026	.....
0.0026	0.0028	0.0037	0.0024	0.0001
0.0026	0.0028	0.0039	0.0025	0.0002
0.0026	0.0056	0.0039	0.0025	0.0002
0.0026	0.0056	0.0040	0.0026	0.0003

It will be noted that the added vanadium has in no way interfered with the determination of phosphorous and that the vanadium was not precipitated from the filtrate after filtering off the magnesium ammonium phosphate.

Thinking that perhaps the vanadium might be precipitated only in the presence of the magnesium ammonium phosphate, the experiment was repeated, the magnesium precipitate being allowed to stand 24 hours. The results are given in Table III.

TABLE III

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS, MAGNESIUM PRECIPITATE ALLOWED TO REMAIN 24 HOURS

P_2O_5 Taken gm.	V_2O_5 Added gm.	$Mg_2P_2O_7$, 24 hrs. gm.	P_2O_5, 24 hrs. gm.
0.0043	None	0.0066	0.0042
0.0043	None	0.0069	0.0044
0.0043	0.0014	0.0067	0.0043
0.0043	0.0014	0.0071	0.0045
0.0043	0.0028	0.0067	0.0043
0.0043	0.0028	0.0067	0.0043

It will be noted that the results are not altered by the added vanadium. The ammonium phospho-molybdate was quite deeply colored in those experiments where vanadium was added, but the tests of the filtrates from the magnesium precipitate showed that the vanadium was still in solution in the ammonia. The ignited precipitates had only a faintly yellow tint and showed only traces of vanadium.

To make our position absolutely sure, however, a third series of experiments was undertaken. In this instance the phosphoric acid was determined not only gravimetrically as pyrophosphate, but the yellow precipitate was also weighed on a Gooch crucible as $(NH_4)_3PO_4 \cdot 12\ MoO_3$ after drying at 160° to 180° to constant weight (5, p. 598), and also determined volumetrically according to the method of J. A. Prescott (6). The results are shown in Table IV.

TABLE IV

RESULTS SHOWING THE EFFECT OF VANADIUM UPON THE DETERMINATION OF SOIL PHOSPHOROUS BY THREE DIFFERENT METHODS

P_2O_5 Taken gm.	V_2O_5 Taken gm.	P_2O_5 as $Mg_2P_2O_7$ after 24 hrs. gm.	P_2O_5 as Yellow Ppt. Grav. gm.	P_2O_5 Titration of Yellow Ppt. gm.
0.0038	None	0.0039	0.0037	0.0038
0.0038	None	0.0038	0.0036	0.0038
0.0038	None	0.0036	.....	0.0038
0.0038	0.0014	0.0042	0.0036	0.0038
0.0038	0.0014	0.0040	0.0036	0.0037
0.0038	0.0028	0.0037	0.0036	0.0036
0.0038	0.0028	0.0037	0.0036	0.0036

There is no doubt that some vanadium does come down with the yellow precipitate, but that the amount which does precipitate is only a small part of that in solution was demonstrated by qualitative tests on the filtrate from the molybdate precipitation (cf. Cain and Hostetter, Reference 1).

It is equally well demonstrated that when vanadium is present, the precipitation of phosphorous by ammonium molybdate is interfered with and may be incomplete (2). We have purposely limited ourselves to the phosphorous content present in normal soils, using only soil solutions in order to have all of the usual elements present in their usual quantities, and adding vanadium in such quantity as to considerably exceed the maximum amount which would probably be expected in soils.

Possibly our final results represent, to a slight extent, a balancing of errors, but we believe that we have demonstrated that the earlier determinations of phosphoric acid in rocks and soils do not contain appreciable errors due to the presence of vanadium, and that the vanadium present in the soil will not interfere with either the volumetric or the gravimetric determination of phosphorous, even though the magnesia precipitate should stand for 24 hours.

SUMMARY

Robinson suggests that vanadium, if present in a soil or rock, may be precipitated together with the phosphoric acid by ammonium molybdate and may introduce grave errors in the determination of phosphorous as magnesium pyrophosphate if the magnesium precipitate is allowed to

stand more than 3 to 4 hours. This statement is apparently based upon a single determination.

We have made a series of determinations, using soil solutions to which known amounts of vanadium were added, the phosphorous being weighed as pyrophosphate, after standing for 24 hours, and in no instance do we find any appreciable interference by vanadium. The titrametric method for phosphorous is also unaffected by the presence of vanadium in amounts far in excess of the usual soil content.

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